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| <b>(51) International Patent Classification <sup>6</sup> :</b><br><b>C12N 15/11, 15/29, A01H 3/00, 5/00</b>   | <b>A1</b>   | <b>(11) International Publication Number:</b> <b>WO 96/34949</b><br><b>(43) International Publication Date:</b> 7 November 1996 (07.11.96) |
| <b>(21) International Application Number:</b> PCT/AU96/00264<br><b>(22) International Filing Date:</b> 3 May 1996 (03.05.96)<br><b>(30) Priority Data:</b><br>PN 2834 5 May 1995 (05.05.95) AU<br><b>(71) Applicants (for all designated States except US):</b> COMMONWEALTH SCIENTIFIC AND INDUSTRIAL RESEARCH ORGANISATION [AU/AU]; Limestone Avenue, Campbell, ACT 2601 (AU). THE AUSTRALIAN NATIONAL UNIVERSITY [AU/AU]; Acton, ACT 2601 (AU).<br><b>(72) Inventors; and</b><br><b>(75) Inventors/Applicants (for US only):</b> PRYOR, Anthony, J. [AU/AU]; Commonwealth Scientific and Industrial Research Organisation, Division of Plant Industry, P.O. Box 1600, Canberra, ACT 2601 (AU). ROBERTS, James, K. [US/US]; University of Georgia, Dept. of Crop and Soil Sciences, Athens, GA 30602-7272 (US).<br><b>(74) Agents:</b> SLATTERY, John, Michael et al.; Davies Collison Cave, 1 Little Collins Street, Melbourne, VIC 3000 (AU). | <b>(81) Designated States:</b> AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).<br><br><b>Published</b><br><i>With international search report.</i> |  |

**(54) Title:** PLANT PROMOTER ACTIVATED BY FUNGAL INFECTION**(57) Abstract**

The present invention relates generally to genetic sequences which are useful in the diagnosis and treatment of fungal infections in plants which involve a susceptible interaction between a fungal pathogen and the host plant. In particular, the present invention provides genetic sequences which confer, activate, or enhance expression of a gene in a plant, in response to infection of said plant by a plant fungal pathogen in a susceptible interaction. The invention further provides genetic sequences such as structural genes, the expression of which is induced in response to a susceptible interaction between a plant and a fungal pathogen. The present invention further provides methods for the detection of infection by a fungal pathogen in a susceptible interaction and for the production of transgenic plants with improved resistance to said fungal pathogen. Transgenic plant material which expresses an artificial resistance gene against a fungal pathogen is also within the scope of the invention claimed.

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## PLANT PROMOTER ACTIVATED BY FUNGAL INFECTION

The present invention relates generally to genetic sequences which are useful in the diagnosis and treatment of fungal infections in plants which involve a susceptible interaction  
5 between a fungal pathogen and the host plant. In particular, the present invention provides genetic sequences which confer, activate, or enhance expression of a gene in a plant, in response to infection of said plant by a plant fungal pathogen in a susceptible interaction. The invention further provides genetic sequences such as structural genes, the expression of which is induced in response to a susceptible interaction between a plant and a fungal pathogen. The  
10 present invention further provides methods for the detection of infection by a fungal pathogen in a susceptible interaction and for the production of transgenic plants with improved resistance to said fungal pathogen. The present invention is particularly useful for developing disease resistance in crop varieties.

15 Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated integer or group of integers but not the exclusion of any other integer or group of integers.

20 Bibliographic details of the publications referred to by author in this specification are collected at the end of the description. Sequence identity numbers (SEQ ID NOs.) for the nucleotide and amino acid sequences referred to in the specification are defined after the bibliography.

25 Advances in plant biotechnology have dramatically altered the approaches taken to increase the economic output of productive units of agriculture. Of major significance to the agricultural and horticultural industries are the reduced productivity, due to infection by plant pathogens. Plant fungal pathogens, in particular rust fungi, represent an especially significant problem amongst broadacre crops such as legume and cereal grains. Biotechnology offers  
30 considerable scope for addressing this problem, by introducing recombinant genes into plants

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that either kill or disable a fungal pathogen, or restrict a fungal pathogen to a limited zone of infection, thereby preventing significant deterioration of an economically-important crop. Thus, the development of disease resistant plants by biotechnological means, is an important goal in agricultural and horticultural research.

5

Genetic analyses indicate that rust resistance genes of the plant genome control specific recognition of the products of rust avirulence genes. An interaction between a rust pathogen and a plant host may be classed as either "resistant" or "susceptible" depending on how the fungal infection proceeds. In a resistant interaction, infection by a fungal pathogen produces  
10 a "plant hypersensitive response" (Marineau *et al.*, 1987; Dixon and Lamb, 1990) resulting in cell death to limit spread of the fungus. During the hypersensitive response, the expression of several infection-related genes, for example genes encoding phytoalexins, antimicrobial agents and pathogenesis-related (PR) proteins, is switched on. In contrast, a susceptible interaction involves no hypersensitive cell death and the infection alters host cell gene expression in such  
15 a way as to provide gene products that are essential for the biotrophic growth of an obligate plant pathogen. Thus, the two processes are quite distinct, involving different host cell genes and mechanisms regulating the expression of said host cell genes. This distinction is of paramount importance. For example, those host genes induced in a susceptible interaction may be essential to allow the rust to grow in the plant tissues.

20

Most studies have concentrated on identifying and manipulating genes encoding proteins involved in the hypersensitive response of the resistant interaction (Collinge and Slusarenko, 1987; Dixon and Lamb, 1990; Keen, 1992; van Loon, 1985; Ohashi and Ohshima, 1992). Marlini and Strittmatter (Patent Application WO 9319188) have constructed a fungus-  
25 responsive chimaeric gene, using a promoter sequence from the *prp1* gene, in particular the *prp1-1* gene, to direct expression of a "killer" gene in plant cells infected by a fungal pathogen. However the *prp1* genetic sequence is induced in a resistant interaction only (i.e. in a pathogenesis-related fungal infection). Although genetic sequences such as the *prp1* gene may provide a means of control of a pathogen in a resistant interaction, the isolation of host cell  
30 genetic sequences involved in a susceptible interaction between a plant and a fungal pathogen

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has not been a straightforward procedure.

In full-susceptible interactions between the flax plant *Linum usitatissimum* and the flax rust *Melampsora lini*, there is no immediate host cell death or chlorosis. Instead, host cell metabolism is directed toward the production of viable fungal spores, including for example, the translocation of photosynthates *via* the haustorium or fungal absorptive organ to the fungal mycelium. Although altered patterns of protein synthesis have been observed following a susceptible rust infection of flax plants (Sutton and Shaw, 1986), it has not been possible, until the present invention, to differentiate between fungal protein synthesis and modifications to plant protein synthesis. Thus, the isolation of flax genetic sequences, the expression of which is induced during a susceptible rust infection, has not been a straightforward procedure.

In work leading up to the present invention, the inventors sought to develop plants with improved resistance to fungal pathogens which would otherwise infect the plant in a susceptible interaction. Accordingly, the inventors identified plant genetic sequences in flax and maize, the expression of which increases in response to a susceptible rust infection. The cloning of these sequences provides a means of generating transgenic plants with *de novo*, improved, or otherwise enhanced antifungal properties. In particular, by placing a cytotoxic gene, anti-fungal gene, antisense, ribozyme or co-suppression molecule operably under control of a promoter sequence derived from a genetic sequence which is normally transcriptionally up-regulated in response to a susceptible infection, and introducing the resulting chimeric gene into a plant, disease resistance against said fungal pathogen is conferred or otherwise facilitated in said plant. The present invention also permits the screening, through genetic or immunological means, similar susceptible reaction-responsive (SRR) genetic sequences in other plants, for use in developing or enhancing the antifungal properties of commercially- and economically-important species.

Accordingly, one aspect of the present invention comprises an isolated nucleic acid molecule comprising a sequence of nucleotides which is capable of conferring, activating, enhancing or otherwise increasing the expression of a structural gene in response to a

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susceptible interaction between a host plant and a fungal pathogen.

Hereinafter the term "susceptible reaction-responsive promoter" or "SRR promoter", or similar term shall be used to define a nucleic acid molecule which is capable of activating or  
5 increasing the expression of a structural gene following interaction between a host plant or host plant cell and a fungal pathogen capable of producing a susceptible infection in said host.

Reference herein to a "promoter" is to be taken in its broadest context and includes the *cis*-regulatory sequences of a classical genomic gene, including the TATA box which is  
10 required for accurate transcription initiation, with or without a CCAAT box sequence and additional regulatory elements (i.e. upstream activating sequences, enhancers and silencers) which alter gene expression in response to developmental and/or environmental stimuli, or in a tissue-specific manner. A promoter is usually, but not necessarily, positioned upstream or 5', of a structural gene, the expression of which it regulates. Furthermore, the regulatory elements  
15 comprising a promoter are usually positioned within 2 kb of the start site of transcription of the gene.

In the present context, the term "promoter" is also used to describe a synthetic or fusion molecule, or derivative which confers, activates or enhances expression of a structural gene or  
20 other nucleic acid molecule, in response to a susceptible interaction between a host and a fungal pathogen. Preferred SRR promoters may contain additional copies of one or more specific regulatory elements to further enhance expression following fungal infection, and/or to alter the time taken between infection and the enhanced expression, the only requirement being that the SRR promoters are derived from naturally-occurring SRR promoters by standard recombinant  
25 techniques.

Generally, an SRR promoter sequence may be subjected to mutagenesis to produce single or multiple nucleotide substitutions, deletions and/or additions. Nucleotide insertional derivatives of the SRR promoter sequence of the present invention include 5' and 3' terminal  
30 fusions as well as intra-sequence insertions of single or multiple nucleotides. Insertional

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nucleotide sequence variants are those in which one or more nucleotides are introduced into a predetermined site in the nucleotide sequence although random insertion is also possible with suitable screening of the resulting product. Deletional variants are characterised by the removal of one or more nucleotides from the sequence. Substitutional nucleotide variants are those in which at least one nucleotide in the sequence has been removed and a different nucleotide inserted in its place.

The present invention therefore, is directed primarily to the SRR promoter sequences of a classical genomic gene the expression of which is up-regulated in response to a susceptible interaction between a host plant and a fungal pathogen, wherein said SRR promoter sequences confer or activate high-level expression on the gene as a result of said susceptible interaction. It will also be known to those skilled in the art that the activation of gene expression which is observed following a susceptible interaction between a host plant and a fungal pathogen is the result of an interaction between said SRR promoter sequences and any number of cell-specific *trans*-acting transcription factors. However, the present invention lies in the nucleic acid molecule which comprises said SRR promoter sequences which are particularly useful in conferring high-level expression in a plant cell, on any structural gene to which it is operably linked, wherein the activated expression is in response to a susceptible interaction. For the successful performance of the present invention, it is not necessary that the *trans*-acting transcription factors are isolated or even known, merely that a plant cell carrying the nucleic acid molecule of the invention produces said factors.

As used herein, the term "structural gene" shall be taken in its broadest context to refer to the transcribed portion of a gene comprising a DNA segment encoding a protein, polypeptide or a portion thereof and includes introns, exons, 5' and 3' untranslated regions of a gene. A structural gene may constitute an uninterrupted coding sequence or it may include one or more introns, bounded by the appropriate plant-functional splice junctions. A structural gene may be a composite or segments derived from a plurality of sources, naturally occurring or synthetic. A structural gene may also encode a fusion protein.

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Expression of a structural gene in a cell is regulated by the *cis*-regulatory sequences to which it is operably linked. The *cis*-regulatory sequence may be a homologous or heterologous sequence, relative to the structural gene.

5       A homologous *cis*-regulatory sequence is one which is operably linked to a particular structural gene in the cell from which it was originally isolated, without any genetic manipulation having been performed thereon. A classical genomic gene, therefore, comprises a homologous *cis*-regulatory sequence operably linked to a structural gene.

10       Hereinafter, the term "genetically-linked *in vivo* to a structural gene" shall be taken to define such homologous *cis*-regulatory sequences.

A heterologous *cis*-regulatory sequence is one which is operably linked to a structural gene other than the structural gene to which it would be linked in the absence of human  
15 intervention. When a heterologous *cis*-regulatory sequence is operably linked to a structural gene, the resulting gene is usually termed a "chimeric gene".

Those skilled in the art are aware that the percentage nucleotide sequence identity between homologous genomic genes isolated from different species is highest within the region  
20 comprising the structural gene, in particular the coding region thereof. Furthermore, although the *cis*-regulatory sequences of such homologous genomic genes may possess some nucleotide sequence identity, the regions of highest identity are usually limited to short stretches of approximately 6-10 nucleotides in length. However, features of secondary structure in the *cis*-regulatory sequences as a whole, which may be as long as 2.5 kilobases, may contribute to the  
25 overall regulatory activity of any particular *cis*-regulatory sequence.

A preferred embodiment of the present invention, provides an isolated nucleic acid molecule which is capable of conferring or activating expression on a structural gene in response to a susceptible interaction between a plant cell and a fungal pathogen, wherein said  
30 nucleic acid molecule is genetically-linked *in vivo* to a structural gene which is at least 40%

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identical to the nucleotide sequence set forth in SEQ ID NO:1 or a complementary strand thereof.

Preferably, the percentage similarity to the sequence set forth in SEQ ID NO: 1 is at least 40%. More preferably, the percentage similarity is at least 60-65%. Still more preferably, the percentage similarity is at least 70-75%. Even more preferably, the percentage similarity is at least 80-90%, including at least 91% or 93% or 95%.

For the purposes of nomenclature, the nucleotide sequence set forth in SEQ ID NO:1 is the flax *FisI* structural gene. The flax *FisI* genomic gene was normally expressed in response to a susceptible interaction between the flax plant *Linum usitatissimum* and the flax rust *Melampsora lini* (Table 1), for example between *L.usitatissimum* cv. Hoshangabad and *M. lini* CH5, as described in Example 1 described herein.

In an alternative embodiment, the present invention provides an isolated nucleic acid molecule which is capable of conferring or activating expression on a structural gene in response to a susceptible interaction between a plant cell and a fungal pathogen, wherein said nucleic acid molecule is genetically-linked *in vivo* to a structural gene which hybridises under at least low stringency conditions to the nucleotide sequence set forth in SEQ ID NO:1 or a complementary strand thereof.

For the purposes of defining the level of stringency, a low stringency is defined herein as being a hybridisation and/or a wash carried out in 6xSSC buffer, 0.1% (w/v) SDS at 28°C. Generally, the stringency is increased by reducing the concentration of SSC buffer, and/or increasing the concentration of SDS and/or increasing the temperature of the hybridisation and/or wash. Conditions for hybridisations and washes are well understood by one normally skilled in the art. For the purposes of clarification, (to parameters affecting hybridisation between nucleic acid molecules), reference is found in pages 2.10.8 to 2.10.16. of Ausubel *et al.* (1987), which is herein incorporated by reference.

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More preferably, the present invention provides an isolated nucleic acid molecule which is capable of conferring or activating expression on a structural gene in response to a susceptible interaction between a plant cell and a fungal pathogen, wherein said nucleic acid molecule:

- 5 (i) is genetically-linked *in vivo* to a structural gene which hybridises under at least low stringency conditions to the nucleotide sequence set forth in SEQ ID NO:1 or a complementary strand thereof; and
- (ii) is genetically-linked *in vivo* to a structural gene which is at least 40% identical to the nucleotide sequence set forth in SEQ ID NO:1 or a complementary strand thereof.

10 In a particularly preferred embodiment of the invention, there is provided an isolated nucleic acid molecule which is capable of conferring or activating expression on a structural gene in response to a susceptible interaction between a plant cell and a fungal pathogen, wherein said nucleic acid molecule comprises a sequence of nucleotides of at least 2 kb in length, wherein said sequence includes at the 5'-end the sequence of nucleotides set forth in  
15 SEQ ID NO:3 or a homologue, analogue or derivative thereof and at the 3'-end the sequence of nucleotides set forth in SEQ ID NO:4 or a homologue, analogue or derivative thereof.

For the purposes of nomenclature, the sequence shown in SEQ ID NO: 3 and/or SEQ ID NO: 4 relate to the 5' and 3' ends of the *Fis 1* gene promoter isolated from flax, which is  
20 capable of inducing expression of the flax *Fis 1* gene following infection with flax rust, in a susceptible interaction. The complete nucleotide sequence of the *Fis 1* promoter, intervening between SEQ ID NO: 3 and SEQ ID NO: 4, is contained in a single 2.2 kb DNA fragment, as described in Example 5 below. The utility of this nucleic acid molecule is exemplified in, but not limited to, the disclosures of Examples 7, 8, and 9 below.

25

More particularly preferred, the isolated nucleic acid molecule of the invention comprises a sequence of nucleotides which is capable of hybridising under at least low stringency conditions to the SRR promoter sequence of the flax *Fis1* gene contained in the microorganism deposited under AGAL Accession No. N96/027087.

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Optionally, the isolated nucleic molecule according to these particularly preferred embodiments of the invention is operably linked to a structural gene.

Those skilled in the art will be aware that the expression of any structural gene may be  
5 operably linked to the isolated nucleic acid molecule of the invention and as a consequence, the scope of the present invention extends to the use of any structural gene, the expression of which is desired to be increased in response to a susceptible infection by a fungal pathogen.

In a most particularly preferred embodiment of the present invention, the structural gene  
10 is a reporter gene selected from the list comprising the bacterial  $\beta$ -glucuronidase (*uidA* or *GUS*) and chloramphenicol acetyltransferase genes and the firefly luciferase gene, amongst others or a cytotoxin gene for example the Barnase gene or other gene encoding a ribonuclease enzyme, amongst others.

15 Cytotoxin genes are well-known to those skilled in the art, as are the methods required for the genetic manipulation and expression in transgenic plants. Whilst not being bound by any theory or mode of action, wherein the structural gene encodes a cytotoxin molecule, the expression of said structural gene in a plant under the control of the SRR promoter sequences of the invention, will be induced in response to a susceptible interaction between said plant and  
20 a fungal pathogen, thereby killing the plant cells at the site of infection and preventing the spread of the fungal pathogen to other sites on the same plant or to neighbouring plants.

The invention further contemplates the use of the SRR promoter sequence of the invention to control the expression of a nucleotide sequence encoding an antisense or a  
25 ribozyme molecule, wherein said ribozyme or antisense molecule is capable of inhibiting the expression of a gene required for viability of a plant cell. According to this embodiment, the expression of said antisense or ribozyme molecule will kill the cells which have been infected by an invading fungal pathogen in a susceptible interaction between said pathogen and plant, thereby preventing the continued growth or reproduction of the fungal pathogen in the infected  
30 plant, analogous to the mode of action of a cytotoxin gene placed operably under the control

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of an SRR promoter sequence.

The invention further contemplates the use of the SRR promoter sequence of the invention to control the expression of a nucleotide sequence encoding a ribozyme, antisense or  
5 co-suppression molecule, wherein said ribozyme, antisense or co-suppression molecule is capable of inhibiting the expression of a fungal gene, for example a fungal gene which is required for fungal growth or reproduction. According to this embodiment, the expression of said antisense or ribozyme molecule will prevent the continued growth or reproduction of the fungal pathogen in the infected plant following a susceptible interaction, thereby conferring some degree of  
10 protection on the host plant.

Ribozymes are synthetic RNA molecules which comprise a hybridising region complementary to two regions, each of at least 5 contiguous nucleotide bases in the target mRNA. In addition, ribozymes possess highly specific endoribonuclease activity, which  
15 autocatalytically cleaves the target mRNA. A complete description of the function of ribozymes is presented by Haseloff and Gerlach (1988) and contained in International Patent Application No. WO89/05852. The present invention extends to ribozymes which target an mRNA encoding an SR gene product.

20 Antisense molecules are nucleic acid molecules which are capable of forming a double-stranded duplex with an mRNA molecule, thereby preventing its translation into a polypeptide.

Co-suppression is the reduction in expression of an endogenous gene that occurs when one or more copies of said gene, or one or more copies of a substantially similar gene are  
25 introduced into the cell.

The present invention is particularly directed to an SRR promoter, preferably the flax *Fis 1* gene promoter, that induces host gene expression in a susceptible interaction between the flax plant *Linum usitatissimum* and the flax rust *Melampsora lini*. Examples of flax SRR  
30 promoter sequences that induce gene expression in a susceptible interaction with a flax rust,

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include, but are not limited to, the flax *Fis 1* gene promoter sequence.

The subject invention clearly contemplates other sources of SRR promoter sequences, such as but not limited to the cereal plants wheat, maize, barley, rye, oats and rice amongst  
5 others. According to this embodiment of the present invention, non-limiting examples of potential sources of SRR promoter sequences and the susceptible host/rust interactions in which they induce gene expression, is presented in Table 1.

In a particularly preferred embodiment, the present invention extends to the SRR  
10 promoter sequence from the maize *Mis1* gene which is induced in the susceptible interaction between maize and the maize rust *Puccinia sorghi*, in particular between maize and *P. sorghi* race 1.

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**TABLE 1:** Examples of susceptible interactions between plant species and their respective fungal pathogens

|    | <b>PATHOGEN</b>                             | <b>HOST PLANT</b>       |
|----|---|-------------------------|
| 5  | <i>Puccinia graminis</i>                    | wheat, barley and rye   |
|    | <i>Puccinia striiformis</i>                 | wheat and rye           |
|    | <i>Puccinia recondita</i>                   | rye and wheat           |
|    | <i>Puccinia hordei</i>                      | barley                  |
|    | <i>Puccinia coronata</i>                    | oat                     |
| 10 | <i>Puccinia sorghi</i>                      | maize                   |
|    | <i>Puccinia polysora</i>                    | maize                   |
|    | <i>Puccinia purpurea</i>                    | sorghum                 |
|    | <i>Puccinia sacchari</i>                    | sugar cane              |
|    | <i>Puccinia kuehnii</i>                     | sugar cane              |
| 15 | <i>Puccinia arachidis</i>                   | peanut                  |
|    | <i>Puccinia stachmanii</i>                  | cotton                  |
|    | <i>Uromyces striatus medicaginis</i>        | alfalfa                 |
|    | <i>Uromyces phaseoli</i>                    | <i>phaseolus</i> beans  |
|    | <i>Hemileia vastatrix</i>                   | coffee rust             |
| 20 | <i>Melampsora lini</i>                      | flax                    |
|    | <i>Gymnosporangium juniperi-virginianae</i> | cedar and apple         |
|    | <i>Cronartium ribicola</i>                  | white pine              |
|    | <i>Cronartium fusiforme</i>                 | loblolly and slash pine |

25

The genetic sequences comprising an SRR promoter sequence may correspond to the naturally-occurring sequence, or may differ by one or more nucleotide substitutions, deletions and/or additions.

30

It is understood in the art that modifications may be made to the structural arrangement

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of specific enhancer and promoter elements of the SRR promoter molecule described herein without destroying the improved enhancing activity of gene expression. For example, it is contemplated that a substitution may be made in the choices of plant-expressible enhancer and promoter elements without significantly affecting the function of the recombinant SRR  
5 promoter molecule of this invention. Further, it is contemplated that nucleotide sequences homologous to the active enhancer elements utilized herein may be employed advantageously, either as a substitution or an addition to the recombinant promoter construct for improved gene expression in plant cells, in response to infection of said plant cell with a rust fungus, in a susceptible interaction. It will also be understood by one normally skilled in the art that the  
10 function of an SRR promoter sequence also results from the arrangement, orientation and spacing of the difference enhancer elements with respect to one another, and with respect to the position of the TATA box. Accordingly, the present invention extends to SRR promoter sequences and any functional promoters, derivatives, parts, fragments, homologues, or analogues thereof, or non-functional molecules which are at least useful as, for example genetic  
15 probes in the isolation of similar sequences, or primer sequences in the enzymatic or chemical synthesis of said gene, or in the generation of immunologically interactive recombinant molecules.

The SR structural genetic sequence is particularly useful in isolating the homologous  
20 SRR promoter to which it is operably linked *in vivo*. Thus, by using the SR structural gene as a probe, or to design PCR primers, it is possible to isolate SRR promoter sequences which fall within the scope of the present invention. For example, the inventors have used the *Fis1* promoter sequence to isolate the corresponding maize *Mis1* gene, *via* the *Fis1* structural gene intermediate, as described in Example 11 incorporated herein.

25

The present invention clearly extends to a genetic sequence comprising at least, the coding region of a gene, hereinafter defined as a "susceptible reaction structural gene", or "SR structural gene" which, in its native state is expressed in response to a susceptible interaction between a host and a rust fungus. Reference herein to "SR structural genes" is to be taken in its  
30 broadest context and includes:

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- (i) a classical genomic SR structural gene consisting of transcriptional and/or translational regulatory sequences and/or a coding region and/or non-translated sequences (i.e. introns, 5'- and 3'- untranslated sequences); or
- (ii) mRNA or cDNA corresponding to the coding regions (i.e. exons) and 5'- and 3'-  
5 untranslated sequences of the gene.

The term "SR structural gene" is also used to describe synthetic or fusion molecules encoding all or part of a functional product. Preferred SR structural genes may be derived from a naturally-occurring SR structural gene by standard recombinant techniques. Generally, an SR  
10 structural gene may be subjected to mutagenesis to produce single or multiple nucleotide substitutions, deletions and/or additions. Nucleotide insertional derivatives of the SR structural gene of the present invention include 5' and 3' terminal fusions as well as intra-sequence insertions of single or multiple nucleotides. Insertional nucleotide sequence variants are those in which one or more nucleotides are introduced into a predetermined site in the nucleotide  
15 sequence although random insertion is also possible with suitable screening of the resulting product. Deletional variants are characterised by the removal of one or more nucleotides from the sequence. Substitutional nucleotide variants are those in which at least one nucleotide in the sequence has been removed and a different nucleotide inserted in its place. Such a substitution may be "silent" in that the substitution does not change the amino acid defined by  
20 the codon. Alternatively, substituents are designed to alter one amino acid for another similar acting amino acid, or amino acid of like charge, polarity, or hydrophobicity.

Accordingly, a second aspect of the present invention is directed to an isolated nucleic acid molecule which comprises an SR structural gene as hereinbefore defined.

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Preferably, said SR structural gene comprises a sequence of nucleotides which corresponds or is complementary to the sequence as set forth in SEQ ID NO: 1, or having at least about 40%, more preferably at least about 55%, still more preferably at least about 65%, yet still more preferably at least about 75-80% and even still more preferably at least about 85-  
5 95% nucleotide similarity to all, or a homologue, analogue or derivative thereof.

In an alternative embodiment of this aspect of the invention, said nucleic acid molecule is capable of hybridising under at least low stringency conditions to the nucleic acid molecule set forth in SEQ ID NO: 1, or to a complementary strand thereof.

10

According to this aspect, said SR structural gene encodes, or is complementary to a sequence encoding, a polypeptide which is expressed in response to a susceptible interaction between a host and a rust fungus.

15 In a particularly preferred embodiment, said SR structural gene is the flax *FisI* gene set forth in SEQ ID NO:1 which encodes the amino acid sequence set forth in SEQ ID NO:2 or a homologue, analogue or derivative thereof.

The SR structural gene is particularly useful as a molecular tag in the identification of  
20 homologous nucleic acid molecules which are expressed in response to a susceptible infection by a fungal pathogen. This attribute of the SR structural gene arises from the fact that it is normally expressed under the control of an SRR promoter sequence, for example the flax *FisI* promoter sequence and, as a consequence, expression of the homologous sequence is up-regulated in the cell in response to said fungal infection. In this regard, the SR structural gene  
25 is useful in the detection of increased expression of any SR structural gene to which it is capable of hybridising or to which at least 10 contiguous nucleotides derived therefrom are capable of hybridising under at least low stringency conditions.

Accordingly, the present invention provides, in a further aspect, a method of detecting  
30 infection of a plant by a fungal pathogen which is capable of forming a susceptible interaction

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therewith, said method comprising the step of detecting increased expression of an SR structural gene in said plant.

Preferably, said step of detecting increased expression is performed by contacting a  
5 nucleic acid molecule derived from said plant with a second nucleic acid molecule comprising the nucleotide sequence set forth in SEQ ID NO:1 or a fragment of at least 10 contiguous nucleotides in length derived therefrom for a time and under conditions sufficient to allow a double-stranded nucleic acid molecule to form.

10 More preferably, the second nucleic acid molecule is labelled with a reporter molecule such as a radioactive or biotinylated molecule, enzyme, antibody or any other molecule which can be assayed by known methods. According to this embodiment, the amount of bound nucleic acid molecule formed during the hybridisation step can be assayed by determining the amount of bound reporter molecule.

15

The method according to this aspect of the invention is particularly applicable to the detection of an infection in a plant by a fungal pathogen, wherein said plant and respective fungal pathogen is selected from the list of pathogen: host plant combinations set forth in Table 1.

20

The SRR promoter sequence and/or the SR structural genetic sequence of the present invention are further useful in the isolation of said related SRR promoter sequences and SR structural genes from other plants. Where the level of nucleotide sequence identity between the SRR promoter sequence of the invention and the related sequence is sufficiently high, it is  
25 possible to use the SRR promoter directly as a hybridisation probe. However, where there is insufficient nucleotide sequence similarity between the SRR promoter of the invention and a functionally-related SRR promoter, the related SR structural gene and/or the genomic clone equivalents thereof may be isolated first and used in turn to isolate the related SRR promoter sequence.

30

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According to this embodiment, there is contemplated a method for identifying a related SRR promoter sequence, or SR structural genetic sequence, said method comprising contacting genomic DNA, or mRNA, or cDNA, or parts of fragments thereof, or a source thereof, with a hybridisation-effective amount of a first SRR promoter sequence or first SR structural gene, or  
5 a part thereof, and then detecting said hybridisation.

The related SRR promoter sequence or SR structural genetic sequence may be in a recombinant form, in a virus particle, bacteriophage particle, yeast cell, animal cell, or a plant cell. Preferably, the related genetic sequence originates from *Triticum aestivum* or similar plant  
10 such as barley, rye, oats, maize or rice and/or wild varieties and/or hybrids or derivatives and/or ancestral progenitors of same. In addition, the related genetic sequence may be bound to a support matrix, for example nylon, nitrocellulose, polyacrylamide, agarose, amongst others.

Preferably, the first SRR promoter sequence, or first SR structural genetic sequence, is  
15 from flax, or other plant such as wheat, barley, rye, oats, maize or rice.

Preferably, the SR structural gene comprises a sequence of nucleotides which is capable of hybridising under at least low stringency conditions to the sequence set forth in SEQ ID NO:1 or a complement, homologue, analogue or derivative thereof.  
20

In a particularly preferred embodiment, the SRR promoter sequence comprises a sequence of nucleotides of at least 2 kb in length, wherein said sequence includes at the 5'-end the sequence of nucleotides set forth in SEQ ID NO:3 or a homologue, analogue or derivative thereof and at the 3'-end the sequence of nucleotides set forth in SEQ ID NO:4 or a homologue,  
25 analogue or derivative thereof.

More particularly preferred, the first SRR promoter comprises a sequence of nucleotides which is capable of hybridising under at least low stringency conditions to the SRR promoter sequence of the flax *Fis1* gene contained in the microorganism deposited under AGAL  
30 Accession No. N96/027087.

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Preferably, the SRR promoter sequence, or SR structural gene is labelled with a reporter molecule capable of producing an identifiable signal (eg. a radio isotope such as  $^{32}\text{P}$ , or  $^{35}\text{S}$ , or a biotinylated molecule) to facilitate its use as a hybridisation probe in the isolation of related SRR promoter sequences and SR structural genes.

5

For the purposes of defining the level of stringency, a low stringency is defined herein as being a hybridisation and/or a wash carried out in 6xSSC buffer, 0.1% (w/v) SDS at 28°C. Generally, the stringency is increased by reducing the concentration of SSC buffer, and/or increasing the concentration of SDS and/or increasing the temperature of the hybridisation and/or wash. Conditions for hybridisations and washes are well understood by one normally skilled in the art. For the purposes of clarification, (to parameters affecting hybridisation between nucleic acid molecules), reference is found in pages 2.10.8 to 2.10.16. of Ausubel *et al.* (1987), which is herein incorporated by reference.

15 Alternatively, an SR structural gene and/or the genomic clone equivalent thereof which is related to the flax *Fis1* structural gene set forth in SEQ ID NO:1 may be isolated by amplification using the polymerase chain reaction (PCR) employing oligonucleotide primers derived from SEQ ID NO:1. The polymerase chain reaction procedure used in the present invention involves hybridising a nucleic acid primer molecule of at least 10 nucleotides in  
20 length to a nucleic acid "template molecule", said template molecule herein defined as a SRR promoter sequence, or SR structural genetic sequence, or a functional part thereof, or its complementary sequence. A related SRR promoter may subsequently be isolated, either directly from said genomic clone equivalent or alternatively, by hybridisation using the related SR structural gene as a hybridisation probe. Such methods are well-known to those skilled in the  
25 art.

The nucleic acid primer molecule or molecule effective in hybridisation may be contained in an aqueous mixture of other nucleic acid primer molecules or in a substantially pure form.

30

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In a preferred embodiment, the nucleic acid primer molecule comprises at least 10 contiguous nucleotides in length derived from the from a flax *FisI* sequence set forth in any one of SEQ ID Nos: 1, 3, 4, 5, 6 or 7. For the purposes of nomenclature, SEQ ID NOs: 5-7 each comprise a degenerate sequence of nucleotides which encode or are complementary to a  
5 sequence of nucleotides which encode an SR gene product motif set forth in Table 2.

More preferably, said oligonucleotide molecule comprises a sequence of nucleotides substantially the same as the nucleotide sequence set forth in SEQ ID NO:5, SEQ ID NO:6 or SEQ ID NO:7 or a homologue, analogue or derivative thereof.

10

The nucleic acid template molecule may be in a recombinant form, in a virus particle, bacteriophage particle, yeast cell, animal cell, or a plant cell. Preferably, the related genetic sequence originates from *Triticum aestivum* or similar plant such as barley, rye, oats, maize, or rice and/or wild varieties and/or hybrids or derivatives and/or ancestral progenitors of same.

15

In a particularly preferred embodiment, the present invention provides an oligonucleotide molecule which is useful as a hybridisation probe or PCR primer derived from the nucleotide sequence set forth in any one of SEQ ID Nos: 1, 3 or 4 or a homologue, analogue or derivative thereof.

20

More preferably, said oligonucleotide molecule comprises a sequence of nucleotides substantially the same as the nucleotide sequence set forth in SEQ ID NO:5, SEQ ID NO:6 or SEQ ID NO:7 or a homologue, analogue or derivative thereof.

25

A further aspect of the present invention is directed to a genetic construct comprising an SRR promoter sequence or a homologue, analogue or derivative thereof as hereinbefore defined.

The SRR promoter sequence or a functional derivative, part, fragment, homologue, or  
30 analogue thereof may be used to regulate the expression of a heterologous structural gene such

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as a reporter gene or gene encoding a cytotoxin or alternatively, it may regulate the expression of a nucleic acid molecule which encodes a ribozyme or antisense molecule.

Placing a structural gene under the regulatory control of an SRR promoter means  
5 positioning the structural gene such that the expression of the gene is controlled by these promoter sequences. Promoters are generally positioned 5' (upstream) to the genes that they control. In the construction of heterologous promoter/structural gene combinations it is generally preferred to position the promoter at a distance from the gene transcription start site that is approximately the same as the distance between that promoter and the gene it controls  
10 in its natural setting, i.e., the gene from which the promoter is derived. As is known in the art, some variation in this distance can be accommodated without loss of promoter function. Similarly, the preferred positioning of a regulatory sequence element with respect to a heterologous gene to be placed under its control is defined by the positioning of the element in its natural setting, i.e., the genes from which it is derived. Again, as is known in the art and  
15 demonstrated herein with multiple copies of regulatory elements, some variation in this distance can occur.

Preferred reporter genes include the  $\beta$ -glucuronidase gene, chloramphenicol acetyl transferase gene or the firefly luciferase gene, amongst others. Preferred cytotoxin genes  
20 include the barnase gene or other ribonuclease gene.

The cytotoxin gene or ribozyme or antisense molecule may be any of those discussed *supra* which, when produced in a plant cell either kills, disables or repels a fungus, or kills or at least significantly alters host cell metabolism to limit spread and/or development of said  
25 fungus.

Preferably, the SRR promoter sequence comprises a sequence of nucleotides of at least 2 kb in length, wherein said sequence includes at the 5'-end the sequence of nucleotides set forth in SEQ ID NO:3 or a homologue, analogue or derivative thereof and at the 3'-end the  
30 sequence of nucleotides set forth in SEQ ID NO:4 or a homologue, analogue or derivative

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thereof.

More particularly preferred, the first SRR promoter comprises a sequence of nucleotides which is capable of hybridising under at least low stringency conditions to the SRR promoter  
5 sequence of the flax *Fis1* gene contained in the microorganism deposited under AGAL Accession No. N96/027087.

In a most particularly preferred embodiment, the genetic construct of the present invention comprises the GUS reporter gene operably linked to the flax *Fis1* promoter sequence,  
10 for example in the pFisGUS52 genetic construct set forth in Figure 2a and deposited under AGAL Accession No. N96/027087.

A further aspect of the present invention contemplates a transgenic plant such as a crop plant, carrying a non-endogenous SRR promoter sequence and/or an SR structural genetic  
15 sequence as hereinbefore defined. Preferably the SRR promoter sequence or the SR structural genetic sequence are essentially identical to, or derived from, the flax *Fis 1* nucleotide sequence.

Preferably, the transgenic plant is a flax plant. More preferably, the transgenic plant is  
20 one or more of the following: flax, wheat, barley, oats, rye, rice, maize, amongst others. Additional species are not excluded.

In a most particularly preferred embodiment, the transgenic plant is a flax plant which has been transformed with the genetic construct deposited under AGAL Accession No.  
25 N96/027087.

Methods for the transformation of plant tissue are well-known to those skilled in the art. The technique used for a given plant species or specific type of plant tissue depends on the known successful techniques. Means for introducing recombinant DNA into plant tissue  
30 include, but are not limited to, transformation (Paszkowski *et al.*, 1984), electroporation

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(Fromm *et al.*, 1985), or microinjection of the DNA (Crossway *et al.*, 1986) or T-DNA-mediated transfer from *Agrobacterium* to the plant tissue. Representative T-DNA vector systems are described in the following references: An *et al.* (1985); Herrera-Estrella *et al.* (1983 a,b); Herrera-Estrella *et al.* (1985). Once introduced into the plant tissue, the expression  
5 of the structural gene may be assayed in a transient expression systems, or it may be determined after selection for stable integration within the plant genome. Techniques are known for the *in vitro* culture of plant tissue, and for regeneration into whole plants. Procedures for transferring the introduced gene from the originally transformed plant into commercially useful cultivars are known to those skilled in the art.

10

The present invention extends to the progeny derived from said transgenic plant.

A further aspect of the invention provides for the expression of an SR structural gene in a suitable host (eg a prokaryotic or eukaryotic cell) to produce a full-length or non-full-length  
15 recombinant SR gene product.

Preferably the SR gene product further contains two, still more preferably three and even still more preferably four of the amino acid sequence motifs comprising the list set forth in Table 2. Preferably, the SR gene product has a sequence that is at least 40% identical to the  
20 amino acid sequence set forth in SEQ ID NO: 2 or a homologue, analogue or derivative thereof. In a particularly preferred embodiment, the SR gene product comprises a sequence of amino acids which is substantially the same as the flax Fis1 polypeptide sequence set forth in SEQ ID NO:2.

25 The present invention extends also to a synthetic peptide fragment of an SR gene product, preferably the SR gene product set forth in SEQ ID NO: 2.

**TABLE 2:** SR gene product motifs

| <b>MOTIF</b> | <b>AMINO ACID SEQUENCE</b>            |
|--------------|---------------------------------------|
| GPL          | WPFGPVAIITPFNFPLEIPVLQLMGALYMGNKPLLKV |
| 5 GSG        | RMTLFTGSSRVAEKLALDLKGRIKLED           |
| GQG          | DAYACSGQKCSAQSLFMHE                   |
| EEP          | NYELVTKEIFGPFQVVTEYKNSQLPMVLEA        |

10 In an alternative embodiment, the present invention extends to an SR gene product comprising an amino acid sequence motif which is at least 40% similar, or preferably 40-60% similar, or more preferably 60-90% similar, or still more preferably 90-100% similar, to an amino acid sequence motif selected from the list set forth in Table 2.

15 The present invention extends further to a recombinant gene product comprising an amino acid sequence motif selected from the list set forth in Table 2 in any relative combination, or frequency, or a functional derivative thereof, having at least 40% similarity to same.

20 According to this aspect, the recombinant SR gene product of the present invention, or a functional derivative thereof, may be used to produce immunologically interactive molecules, such as antibodies, or functional derivatives thereof, the only requirement being that the recombinant products are immunologically interactive with antibodies to all or part of an SR gene product.

25

Antibodies to a recombinant SR gene product are particularly useful in the screening of plants for the presence of said gene product. Another aspect of the present invention is, therefore, directed to antibodies to a recombinant SR gene product or part or fragment thereof. Such antibodies may be monoclonal or polyclonal and may be selected from naturally occurring  
 30 antibodies to an SR gene product or may be specifically raised to a recombinant SR gene

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product. In the case of the latter, the SR gene product may first need to be associated with a carrier molecule. Alternatively, fragments of antibodies may be used such as Fab fragments. Furthermore, the present invention extends to recombinant and synthetic antibodies and to antibody hybrids. A "synthetic antibody" is considered herein to include fragments and hybrids  
5 of antibodies. The antibodies and/or the recombinant SR gene products of the present invention are particularly useful for the immunological screening of SR gene products in various plants, leading to the isolation of related SRR promoter sequences and SR structural genes.

In one embodiment, specific antibodies are used to screen for SR gene products in  
10 plants. Techniques for the assays contemplated herein are known in the art and include, for example, sandwich assays and ELISA.

It is within the scope of this invention to include any second antibodies (monoclonal, polyclonal or fragments of antibodies) directed to the first mentioned antibodies discussed  
15 above. Both the first and second antibodies may be used in detection assays or a first antibody may be used with a commercially available anti-immunoglobulin antibody. An antibody as contemplated herein includes any antibody specific to any region of a recombinant SR gene product.

20 Both polyclonal and monoclonal antibodies are obtainable by immunisation with a recombinant SR gene product and either type is utilisable for immunoassays. The methods of obtaining both types of sera are well known in the art. Polyclonal sera are less preferred but are relatively easily prepared by injection of a suitable laboratory animal with an effective amount of recombinant SR gene product, or antigenic or immunointeractive parts thereof,  
25 collecting serum from the animal and isolating specific sera by any of the known immunoabsorbent techniques. Although antibodies produced by this method are utilisable in virtually any type of immunoassay, they are generally less favoured because of the potential heterogeneity of the product.

30 The use of monoclonal antibodies in an immunoassay is particularly preferred because

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of the ability to produce them in large quantities and the homogeneity of the product. The preparation of hybridoma cell lines for monoclonal antibody production derived by fusing an immortal cell line and lymphocytes sensitised against the immunogenic preparation can be done by techniques which are well known to those who are skilled in the art (see, for example, 5 Douillard and Hoffman, 1981; Kohler and Milstein, 1975; Kohler and Milstein, 1976).

The presence of an SR gene product in a plant or more commonly plant extract may be accomplished in a number of ways such as by Western blotting and ELISA procedures. A wide range of immunoassay techniques are available as can be seen by reference to US Patent 10 Nos. 4,016,043, 4, 424,279 and 4,018,653. These, of course, includes both single-site and two-site or "sandwich" assays of the non-competitive types, as well as in the traditional competitive binding assays. These assays also include direct binding of a labelled antibody to a target.

Sandwich assays are among the most useful and commonly used assays and are favoured 15 for use in the present invention. A number of variations of the sandwich assay technique exist, and all are intended to be encompassed by the present invention. Briefly, in a typical forward assay, an unlabelled antibody is immobilised on a solid substrate and the sample to be tested brought into contact with the bound molecule. After a suitable period of incubation, for a period of time sufficient to allow formation of an antibody-antigen complex, a second antibody 20 specific to the antigen, labelled with a reporter molecule capable of producing a detectable signal is then added and incubated, allowing time sufficient for the formation of another complex of antibody-antigen-labelled antibody. Any unreacted material is washed away, and the presence of the antigen is determined by observation of a signal produced by the reporter molecule.

25

In this case, the first antibody is raised to a recombinant SR gene product and the antigen is an SR gene product in a plant.

The results may either be qualitative, by simple observation of the visible signal, or may 30 be quantitated by comparing with a control sample containing known amounts of hapten.

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Variations on the forward assay include a simultaneous assay, in which both sample and labelled antibody are added simultaneously to the bound antibody. These techniques are well known to those skilled in the art, including any minor variations as will be readily apparent. In accordance with the present invention the sample is one which might contain an SR gene product and include crude or purified plant extract such as extracts of leaves, roots and stems.

In the typical forward sandwich assay, a first antibody raised against a recombinant SR gene product is either covalently or passively bound to a solid surface. The solid surface is typically glass or a polymer, the most commonly used polymers being cellulose, polyacrylamide, nylon, polystyrene, polyvinyl chloride or polypropylene. The solid supports may be in the form of tubes, beads, discs of microplates, or any other surface suitable for conducting an immunoassay. The binding processes are well-known in the art and generally consist of cross-linking, covalent binding or physically adsorption, the polymer-antibody complex is washed in preparation for the test sample. An aliquot of the sample to be tested is then added to the solid phase complex and incubated for a period of time sufficient (e.g. 2-40 minutes) and under suitable conditions (e.g. 25°C) to allow binding of any antigen present in the sample to the antibody. Following the incubation period, the reaction locus is washed and dried and incubated with a second antibody specific for a portion of the first antibody. The second antibody is linked to a reporter molecule which is used to indicate the binding of the second antibody to the hapten.

An alternative method involves immobilising the target molecules in the biological sample and then exposing the immobilised target to specific antibody which may or may not be labelled with a reporter molecule. Depending on the amount of target and the strength of the reporter molecule signal, a bound target may be detected by direct labelling with the antibody. Alternatively, a second labelled antibody, specific to the first antibody is exposed to the target-first antibody complex to form a target-first antibody-second antibody tertiary complex. The complex is detected by the signal emitted by the reporter molecule.

By "reporter molecule" as used in the present specification, is meant a molecule which,

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by its chemical nature, provides an analytically identifiable signal which allows the detection of antigen-bound antibody. Detection may be either qualitative or quantitative. The most commonly used reporter molecules in this type of assay are either enzymes, fluorophores or radionuclide containing molecules (i.e. radioisotopes) and chemiluminescent molecules.

5

In the case of an enzyme immunoassay, an enzyme is conjugated to the second antibody, generally by means of glutaraldehyde or periodate. As will be readily recognised, however, a wide variety of different conjugation techniques exist, which are readily available to the skilled artisan. Commonly used enzymes include horseradish peroxidase, glucose oxidase, beta-  
10 galactosidase and alkaline phosphatase, amongst others. The substrates to be used with the specific enzymes are generally chosen for the production, upon hydrolysis by the corresponding enzyme, of a detectable colour change. Examples of suitable enzymes include alkaline phosphatase and peroxidase. It is also possible to employ fluorogenic substrates which yield a fluorescent product rather than the chromogenic substrates noted above. In all cases, the  
15 enzyme-labelled antibody is added to the first antibody-hapten complex, allowed to bind, and then the excess reagent is washed away. A solution containing the appropriate substrate is then added to the complex of antibody-antigen-antibody. The substrate will react with the enzyme linked to the second antibody, giving a qualitative visual signal, which may be further quantitated, usually spectrophotometrically, to give an indication of the amount of hapten which  
20 was present in the sample. The term "reporter molecule" also extends to use of cell agglutination or inhibition of agglutination such as red blood cells on latex beads, and the like.

Alternately, fluorescent compounds, such as fluorescein and rhodamine, may be chemically coupled to antibodies without altering their binding capacity. When activated by  
25 illumination with light of a particular wavelength, the fluorochrome-labelled antibody adsorbs the light energy, inducing a state of excitability in the molecule, followed by emission of the light at a characteristic colour visually detectable with a light microscope. As in enzyme immunoassays (EIA), the fluorescent labelled antibody is allowed to bind to the first antibody-hapten complex. After washing off the unbound reagent, the remaining tertiary complex is then  
30 exposed to the light of the appropriate wavelength the fluorescence observed indicates the

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presence of the hapten of interest. Immunofluorescence and EIA techniques are both very well established in the art and are particularly preferred for the present method. However, other reporter molecules, such as radioisotope, chemiluminescent or bioluminescent molecules, may also be employed.

5

#### Microorganism deposits

A host *Escherichia coli* cell transformed with a vector designated pFisGUS52 comprising the flax *Fis1* gene promoter driving GUS reporter gene expression has been  
10 deposited in accordance with the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure, with the Australian Government Analytical Laboratories P.O. Box 385 Pymble, New South Wales 2073, Australia on 3 May, 1996 under Accession No. N96/027087.

15

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The present invention is further described by reference to the following non-limiting Figures and Examples.

In the Figures:

5

**Figure 1** is a photographic representation of a Southern blot hybridisation demonstrating the origin of pFIS1 from the flax genome. Equal amounts (10µg) of flax DNA (lanes 1 to 3) and rust DNA (lanes 4 to 6) were digested with the restriction enzymes, *Bgl*II (lanes 1 and 4), *Eco*RI (lanes 2 and 5) and *Hind*III (lanes 3 and 6). The DNA was transferred  
10 to membrane and probed with pFIS1.

**Figure 2(a)** is a schematic representation of a recombinant DNA molecule comprising the bacterial  $\beta$ -glucuronidase (*uidA*) structural gene placed operably under control of the flax *Fis 1* gene promoter sequence. The hatched area indicates the *Fis 1* promoter. The filled-in  
15 area indicates the  $\beta$ -glucuronidase structural gene.

**Figure 2(b)** is a photographic representation showing GUS reporter gene expression under control of the flax *Fis 1* gene promoter sequence, in leaf cells of a transgenic flax plant carrying the recombinant DNA molecule of Figure 2(a), following infection with *Melampsora*  
20 *lini*. GUS gene expression which appears as intense blue colouration in plant cells, following staining with X-glucuronide, is indicated in the Figure by the dark spotted regions. Original colour prints are available from the applicant upon request.

**Figure 3** is a photographic representation of a northern blot showing the level of *Fis*  
25 *1* mRNA during susceptible rust infection. Each lane contains 20µg of total RNA isolated from germinated rust spores (1), leaves from susceptible flax plant 1 day after inoculation with rust spores (2), 2 days after inoculation (3), leaves 3 days after inoculation (4), 4 days after inoculation (5), 5 days after inoculation (6), 6 days after inoculation (7) and uninfected leaves (8). The arrows indicate the position of the large and small ribosomal RNAs.

30       A)     The filter was hybridised with the coding region of the *Fis 1* structural gene.

- 30 -

B) To demonstrate that approximately equal amounts of RNA were loaded the same filter as in A was hybridised with a flax cDNA, pFCS1 (Flax Constant Sequences) that does not change during infection (note that exposure was 5 times longer than when probed with pFIS1).

C) The same filter hybridised with an anionic peroxidase cDNA cloned (see 5 Materials and Methods for complete description of peroxidase clone).

Figure 4 is a photographic representation of a northern blot showing the level of *Fis 1* mRNA during resistant infections of flax with flax rust. Each lane contains 20µg per lane of total RNA from leaves of a susceptible plant 5 days after inoculation (1) and RNA from leaves 10 of resistant flax, uninfected (2) 1 day after inoculation (3), 2 days (4), 3 days (5) and 4 days (6), and 5 days (7) after inoculation.

A) The filter was hybridised with pFIS1.

B) The same filter hybridised with an anionic peroxidase cDNA demonstrating the induction of this PR protein during the resistant infection.

15 C) The same filter as in A probed with flax cDNA pFCS1 as a control to demonstrate equal loading of each lane.

Figure 5 is a graphical representation showing a comparison of the amino acid sequences of the maize *Mis1* (row 1) and flax *Fis1* (row 3) SR gene product motifs, including 20 the GPL motif, GSG motif, EEP motif and active site incorporating the GQG motif. Row 2 in each case shows conserved amino acid residues, with conservative amino acid substitutions indicated by the plus sign (+).

Figure 6 is a photographic representation of a northern blot showing the induction of 25 mRNA during the five day period following infection of susceptible maize plants with *Puccinia sorghi* race 1. Each lane contains approximately 30µg of total RNA isolated from leaves of uninfected plants (lane 1) or infected plants, harvested 2, 3, 4 or 5 days post-infection (lanes 2 to 5 respectively).

30 Figure 7 is a photographic representation of a Southern blot probed with the *Mis1*

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sequence under standard stringency hybridisation conditions [insert conditions]. Each lane contains approximately 10 $\mu$ g of *Nco*I-digested total genomic DNA isolated from seedlings of barley cv Franklin (lane 1), barley cv Himalaya (lane 2), oats cv Ascencio (lane 3), oats cv Victoria (lane 4), wheat cv Chinese Spring (lane 5), maize cv Tx303 (lane 6) and maize cv  
5 CO159 (lane 7).

Single letter and three letter abbreviations used for amino acid residues in the specification are defined in Table 3.

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TABLE 3

| 5 Amino Acid  | Three-letter<br>Abbreviation | One-letter<br>Symbol |
|---------------|------------------------------|----------------------|
| Alanine       | Ala                          | A                    |
| Arginine      | Arg                          | R                    |
| 10 Asparagine | Asn                          | N                    |
| Aspartic acid | Asp                          | D                    |
| Cysteine      | Cys                          | C                    |
| Glutamine     | Gln                          | Q                    |
| Glutamic acid | Glu                          | E                    |
| 15 Glycine    | Gly                          | G                    |
| Histidine     | His                          | H                    |
| Isoleucine    | Ile                          | I                    |
| Leucine       | Leu                          | L                    |
| Lysine        | Lys                          | K                    |
| 20 Methionine | Met                          | M                    |
| Phenylalanine | Phe                          | F                    |
| Proline       | Pro                          | P                    |
| Serine        | Ser                          | S                    |
| Threonine     | Thr                          | T                    |
| 25 Tryptophan | Trp                          | W                    |
| Tyrosine      | Tyr                          | Y                    |
| Valine        | Val                          | V                    |

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**EXAMPLE 1****PLANT MATERIAL AND RUST STRAINS**

All susceptible infections with the rust strain CH5 were done on 12-day old flax seedlings (*Linum usitatissimum* cv. Hoshangabad). Fresh spores were sprinkled over the seedlings and allowed to germinate overnight at 20°C, in a humidified environment. For resistant infections, flax seedlings (*L. usitatissimum* cv. Forge, Ellis *et al.*, 1992) was used as the host for rust strain CH5.

**EXAMPLE 2****ISOLATION OF RNA AND DNA FROM FLAX SEEDLINGS**

Flax seedling leaves were ground in liquid nitrogen and suspended in 20 mM TRIS pH 7.5, 100 mM NaCl, 2.5 mM EDTA, 1% SDS, 0.5% 2-mercaptoethanol (5ml/g). Protein was removed by extraction three times with phenol:chloroform:isoamyl alcohol. Total nucleic acid was precipitated with an equal volume of iso-propanol, collected by centrifugation and re-suspended in TE (Maniatis *et al.*, 1982). The RNA was precipitated from the DNA and contaminating carbohydrates by the addition of solid NaCl to a final concentration of 3M NaCl. DNA was extracted in a similar procedure but after the precipitation of total nucleic acid the DNA was purified on a CsCl density gradient (Maniatis *et al.*, 1982).

**EXAMPLE 3****ISOLATION OF A SUSCEPTIBLE RESPONSE cDNA**

Total RNA was extracted from flax leaves (cv. Hoshangabad) infected heavily with rust spores, as described in Examples 1 and 2. Poly A+ RNA was purified on oligo d(T) magnetic beads and reverse-transcribed using an oligo d(T) primer molecule containing a *NotI* restriction site (GGCCGATGCGGCCGC(T)<sub>17</sub>) as the primer for cDNA synthesis. The cDNA was enriched for sequences specific to the rust-infected leaves, by subtractive hybridisation to 10µg of biotinylated polyA+ RNA from uninfected flax leaves. The subtraction procedure was

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carried out three times, after which the purified cDNA sequences were tailed at the 3'-termini using dATP and terminal transferase (BRL, Gainsville, FL USA). The cDNA sequences were subsequently amplified in a polymerase chain reaction and cloned into the plasmid pGEM5 (Promega, Madison, WI USA) as *NotI* DNA fragments.

5

Seven unique cDNA clones were isolated, which were expressed at low levels in uninfected flax leaves. The expression of one cDNA clone, pFIS1, was induced by infection of flax leaves with rust spores in a susceptible infection and was therefore deemed to correspond to an SR structural genetic sequence. The nucleotide sequence of the *Fis1* structural  
10 gene is set forth in SEQ ID NO:1.

#### EXAMPLE 4

##### THE SR STRUCTURAL GENE *Fis 1* IS A FLAX GENETIC SEQUENCE

15 To demonstrate that the cDNA pFIS1, of the foregoing Example 3, is a genetic sequence derived from the flax plant and not from the flax rust, DNA was isolated from both the flax and the rust organisms and hybridised to the pFIS1 cDNA insert.

As shown in Figure 1, pFIS1 hybridised to flax DNA and not to rust DNA confirming  
20 that pFIS1 is from a flax gene defined as *fis1*. The hybridisation pattern was simple, suggesting *fis1* is a low copy number gene. Two hybridising bands were present in both *HindIII* and *BglII* digestions. Since the pFIS1 probe used does not contain these restriction enzyme recognition sites, these data are consistent with there being two genes homologous to pFIS1.

25

#### EXAMPLE 5

##### ISOLATION OF THE *Fis 1* GENE PROMOTER

A genomic clone containing a 2.2 kb SRR promoter fragment was isolated, using the SR structural genetic sequence of the foregoing Examples 3 and 4 as a hybridisation probe. A  
30 lambda genomic library was screened using a 5' end probe from the FIS1 cDNA, the *NruI/AccI*

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fragment. Genomic clones which hybridised to this 5' end probe were screened based on their restriction digestion pattern with the enzymes *Bgl* II and *Acc* I, (there is an *Acc* I site at position 295 and a *Bgl* II site at position 1143 in *FIS1* cDNA) and the size of the inserts was compared with that obtained when genomic DNA is cut with *Bgl* II and *Acc* I. In genomic DNA, there are two fragments that hybridise with the 5' end probe. The 6 genomic clones that were isolated were grouped into two classes based on the restriction digestion pattern with *Bgl* II and *Acc* I. The *Bgl* II fragments containing hybridising DNA were subcloned into a plasmid vector, (Bluescript) and partial sequence data was obtained from each end. Sequence analysis (from the internal *Bgl* II site) showed that of the two classes of clones, one was 100% similar to the *Fis1* cDNA and the other was only about 95% similar. One of those that was 100% similar was used to construct the promoter fusion with the *E coli*  $\beta$ -glucuronidase (GUS) gene as described in Example 7 below. The 5' end and 3' end of the *Fis 1* gene promoter have been sequenced and the nucleotide sequences are set forth in SEQ ID NO: 3 and SEQ ID NO: 4, respectively.

15

#### EXAMPLE 6

##### NUCLEOTIDE SEQUENCE ANALYSIS OF THE *Fis 1* GENETIC SEQUENCES

DNA was sequenced using the Applied Biosystems double stranded DNA sequencing system (Perkin-Elmer, CA, USA). The sequence was analyzed with the Wisconsin GCG package.

Sequence data of the *Fis 1* cDNA clone pFIS1 reveals an ATG translation start codon at nucleotide 57 in SEQ ID NO: 1. The longest open reading frame (1653 base pairs) found in pFIS1 had 551 amino acids and a predicted molecular weight of approximately 61 kDa.

A search of the Blocks (v6.0) and Prosite databases (Henikoff and Henikoff, 1991) of conserved protein sequence motifs revealed that the amino acid sequence of the *Fis 1* gene product, set forth in SEQ ID NO: 2, has several conserved sequence motifs that are found in aldehyde dehydrogenases (Hempel *et al.*, 1993). Amino acids residues surrounding Cys-332

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match the consensus for the cysteine active site found in all aldehyde dehydrogenases (Henikoff and Henikoff, 1991). In addition to this "cysteine active site", the Blocks search revealed three other sequence motifs that are also found in aldehyde dehydrogenases, and are shown in Table 2. The search of the Blocks database gave an estimate p-value  $p < 7.8 \times 10^{-7}$  and a "shuffled" score of 1450=99.86th percentile for the aldehyde dehydrogenase blocks. The first such amino acid sequence identified is between Gly-193 and Lys-226 known as the GPL motif. The second is the GSG motif between Phe-276 and Ala-282. The third motif between Lys-435 and Pro-550 is the EEP motif. A comparison of FIS1 GPL motif sequence with the corresponding region of the *E. coli* proline dehydrogenase polypeptide showed that from amino acids 192 to 225 there is 50% similarity (36/71 amino acids) between the two sequences. Similarly, there is 65% and 50% similarity in GSG motif and the EEP motif, respectively, between flax FIS1 and the *E. coli* proline dehydrogenase polypeptide. The relative size of FIS1 protein is also consistent with it being an aldehyde dehydrogenase. A glutamic acid containing sequence, known as the FGS motif which is present in aldehyde dehydrogenases except the subclass of methylmalonate-semialdehyde dehydrogenases was also missing from FIS1.

#### EXAMPLE 7

##### EXPRESSION OF A *Fis 1*/GUS GENETIC CONSTRUCT IN TRANSGENIC PLANTS

The bacterial  $\beta$ -glucuronidase structural gene was placed operably under control of the SRR promoter sequence, corresponding to the flax *Fis 1* gene promoter of the foregoing Examples 5 and 6, as shown in Figure 2a.

The binary plant transformation vector, pBI101 was used as the source of the GUS gene. The pBI101 plasmid was cut with Bam HI and then filled in with *E. coli* DNA polymerase, Klenow fragment, to form a blunt end. This blunt end was then used in a digestion with *Xba* I to give a plasmid with a 5' *Xba* I end and a 3' blunt end (the GUS end). The 2 kb 5' region of the *Fis 1* gene from the *Nru* I site in the gene, to the *Xba* I in the bluescript vector, was then ligated into the pBI101 plasmid. This resulted in the addition of 43 amino acid from the *Fis 1* coding region being added to the GUS protein. The resultant plasmid, designated pFisGUS52

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and shown in Figure 2(a) has been deposited under AGAL Accession No. N96/027087.

The genetic construct was then introduced into flax plants by *Agrobacterium*-mediated transformation procedures known to those skilled in the art. Transgenic plants were selected on 5 kanamycin containing media. The regenerated plants were tested for GUS activity by infiltrating leaves in 10mM phosphate buffer, 0.5mM K ferricyanide, 0.5mM K ferrocyanide, 1mM X-glucuronide, overnight at 37°C. Leaves of T2 transgenic plants were infected with rust for 4 days and then GUS stained to reveal the pattern of expression. As shown in Figure 2(b), the GUS gene is expressed in plant cells infected in a susceptible interaction with a fungal 10 pathogen. Uninfected cells do not show detectable GUS gene expression.

#### EXAMPLE 8

##### ANALYSIS OF FLAX *Fis 1* GENE EXPRESSION DURING A SUSCEPTIBLE RUST INFECTION

15

Flax RNA was electrophoresed using standard denaturing formaldehyde gels (Maniatis *et al.*, 1982) to separate 10 or 20 µg of total RNA. The RNA was then transferred with 20X SSC to Hybond N membranes (Amersham International, UK). DNA probes were labelled with [ $\alpha$ -<sup>32</sup>P]dCTP using the Megaprime system (Amersham). The amounts of <sup>32</sup>P-labelled probe 20 hybridizing to the filters was quantified with a Phosphor Imager (Molecular Dynamics, CA USA), using data from three separate experiments.

The pFIS1 cDNA hybridised to a mRNA in Hoshangabad flax which increased in abundance after infection with the flax rust CH5 (Figure 3A). The increase in pFIS1 mRNA 25 paralleled the development of infection with rust. In the early stage of infection (1 and 2 days post inoculation) the level of RNA is almost unchanged. Later (5-6 days after inoculation) when many mesophyll cells are infected with rust, the level of mRNA homologous to pFIS1 increased 10 fold, consistent with the expected pattern of induction for an SRR structural gene. The data shown in Figure 2b indicate, however, that the number of leaf cells which contribute 30 to this 10-fold overall increase in expression is small, suggesting that the actual level of *Fis1*

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gene induction per cell is much greater than 10-fold. Clearly, the 10-fold induction observed for the whole leaf is subject to a significant dilution effect from the contribution of the vast majority of cells in which no induction occurs.

5       The *Fis 1* mRNA is approximately 1.9 kb in size. To check that there was approximately equal loading of RNA, a low abundance cDNA (pFCS1 Flax Control Sequence) whose mRNA does not change was used to probe the same northern blot (Figure 3B). Slightly less hybridization was detected in leaves 6 days after inoculation. This minor decrease may reflect a dilution effect due to the increase in the proportion of fungal RNA, thus  
10 causing a dilution of plant transcripts.

#### EXAMPLE 9

##### EXPRESSION OF THE SR STRUCTURAL GENE *Fis 1* IS NOT INDUCED IN A RESISTANT RUST INTERACTION

15

To determine whether expression of the SR structural gene *Fis 1* is induced during a resistant rust infection, northern hybridisations were performed on Forge flax infected with the flax rust CH5 as described in the preceding Examples, using the pFIS1 cDNA insert as a hybridisation probe.

20

In the resistant reaction, there was no increase in the observed levels of *fis1* mRNA over a 5 day period (Figure 4A). In contrast, a 5-fold increase (on average) was observed in the level of the control anionic peroxidase mRNA, at the very early stages of the resistant infection (Figure 4B). The pattern of accumulation of *Fis 1* mRNA was unlike the induction pattern of  
25 a gene for a typical PR protein. These data and the data from Example 8 suggest that the SR structural gene, *Fis 1* is regulated by distinct cellular mechanisms to those processes which regulate host cell gene expression during a resistant interaction between said host and a rust fungus.

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## EXAMPLE 10

ISOLATION OF THE MAIZE HOMOLOGUE OF THE FLAX *Fis1* GENE

In order to isolate maize cDNAs similar to the flax cDNA (pFIS1) degenerate  
5 oligonucleotide primers to two regions (the GPL and EEP motifs shown in Table 2) of the  
flax protein (FIS1) were designed. The nucleotide sequences of the digonucleotides used are  
set forth in SEQ ID Nos: 5, 6 and 7.

The fragments were then cloned into the Promega vector pGEM-T utilizing the "A  
10 overhang" added by Taq polymerase.

The expected size of the amplification product is approximately 700bp, based on the  
*Fis1* structural gene sequence. However, the PCR reactions produced at least three additional  
DNA fragments, 2 smaller and one larger than the expected 700 base pair fragment was used  
15 for further experiments because it was assumed to have GPL and EEP motifs in the same  
position as FIS1.

The template cDNA for PCR was synthesized from maize RNA, extracted from 3  
week old maize plants 6 days after a susceptible infection with a maize rust. Total RNA was  
20 treated with Rnase free DNase (DnaseQ) prior to cDNA synthesis, using the conditions  
supplied by, Promega. The reverse transcriptase reactions used 20µg of total RNA and was  
primed with 200µg of oligo d(T) in a 25µl reactions using MMV reverse transcriptase from  
New England Biolabs in the recommended conditions. After the reverse transcription 0.5µl  
of this reactions was used in a 20µl PCR reactions. The PCR conditions were as follows, 10  
25 mM KCl, 10mM Tris pH8.3, 1.5mM MgCl, 2µM each primers, 2mM each dNTP and 1 unit  
of Taq polymerase from Boehringer Mannheim. The cycling parameters were: (94°C, 1 min;  
45°C, 30 sec; 73°C, 2 min) 6X; (94°C, 10 sec; 45°C, 20 sec; 73°C, 2 min) 32X; (94°C,  
20 sec; 45°C, 20 sec; 73°C, 3.5 min) 1X using a Corbett Research thermal cycler. The  
primers were, EEP=5' AAA/g GAA/g ATA/t/c TTT/c GGI CCI TT 3' (SEQ ID NO: 5) and  
30 GPL=5' ATA/t/c ACI CCI TTT/c AAT/c TTT/c CC 3' (SEQ ID NO: 6) (I=inosine).

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The products of the PCR reaction were separated on a TBE, 1 % agarose gel and the 700 base pair fragment was eluted from the gel using the Qiagen gel extract kit.

The 700 base pair PCR product was then sequenced using an ABI automated  
5 sequencer. The sequence was used to search the data bases for similar sequences. The closest match to the maize sequence was that of the flax gene *Fis1*. This gave a blast score of 494, while the next highest score was 90 for a comparison to p5C dehydrogenase (1-pyrroline-5-carboxylate dehydrogenase), an aldehyde dehydrogenase from *Bacillus subtilis*. The maize *Mis1* structural gene is 72% identical over 633 nucleotides to the flax *Fis1*  
10 structural gene at the nucleotide sequence level and 84% identical over 204 amino acids at the amino acid level. A comparison of the derived maize *Mis1* and flax *Fis1* polypeptides is provided in Figure 5.

#### EXAMPLE 11

##### 15 EXPRESSION OF THE MAIZE SR STRUCTURAL GENE *Mis1* IN A SUSCEPTIBLE INTERACTION WITH *Puccinia sorghi* race 1

Maize RNA was electrophoresed using standard denaturing formaldehyde gels (Maniatis  
*et al.*, 1982) to separate 30 µg of total RNA. The RNA was then transferred with 20X SSC to  
20 Hybond N membranes (Amersham International, UK). DNA probes were labelled with [ $\alpha$ -<sup>32</sup>P]dCTP using the Megaprime system (Amersham). The amounts of P-labelled probe hybridizing to the filters was quantified with a Phosphor Imager (Molecular Dynamics, CA USA), using data from three separate experiments.

25 The maize *Mis1* structural gene hybridised to a mRNA which increased in abundance after infection with the maize rust *Puccinia sorghi* race 1 (Figure 6). The increase in *Mis1* mRNA observed is consistent with the expected pattern of induction for an SR structural gene.

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**EXAMPLE 12****IDENTIFICATION OF FURTHER GENES RELATED *FisI* AND *MisI***

To determine whether other cereal crop plant species contain SRR promoters and SR  
5 structural genes related to *FisI* or *MisI*, genomic DNA was isolated from seedlings of barley,  
oats, wheat and maize and transferred to a membrane support and probed with the *MisI* SR  
structural gene sequence.

The data indicate the presence of SR-regulated genes such as *FisI* and *MisI* in all  
10 species examined (Figure 7). Those skilled in the art will be aware that the demonstration of  
such sequences in other plants provides a means for their subsequent isolation

**EXAMPLE 13**

15 **EXPRESSION OF THE BARNASE GENE UNDER CONTROL OF THE *FisI***  
**PROMOTER IN TRANSGENIC PLANTS**

The barnase structural gene is placed operably under control of the *FisI* promoter  
sequence by replacing the GUS gene present in the binary vector described above, in "Example  
20 7" with the coding region of the barnase gene. Such methods are well within the means of those  
ordinarily skilled in the art, without undue experimentation.

The resultant genetic construct comprising the barnase coding region placed operably  
behind the *FisI* promoter is then introduced into Hoshangabad flax plants by *Agrobacterium*-  
25 mediated transformation procedures as described in Example 7. Transgenic plants are selected  
on kanamycin containing media and screened for expression of the barnase gene using  
conventional northern hybridisation, RNase protection or PCR approaches.

The regenerated transformed plants are subsequently infected with a flax rust capable  
30 of forming a susceptible interaction with the host plant, in particular the strain CH5, to

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determine whether the transgenic plants exhibit improved resistance to the flax rust. Control experiments are performed using non-transformed isogenic flax plants of similar age and grown under identical conditions.

- 5        Statistically-significant protection ( $p < 0.05$ ) is afforded the transgenic plants by the expression of the barnase gene under control of the flax *FisI* gene promoter, following a susceptible interaction with *Melampsora lini*. In particular, although infection rates of non-transformed control plants and transformed plants are similar within the first 24hr post-infection, the level of lesions in the transformed plants is markedly reduced within 14 days
- 10 post-infection and the transformed plants recover their vigour rapidly. Thus, the approach of expressing a cytotoxin gene under the control of an SRR promoter sequence such as *FisI* is useful in providing protection against fungal pathogens in a susceptible interaction.

- Those skilled in the art will appreciate that the invention described herein is susceptible
- 15 to variations and modifications other than those specifically described. It is to be understood that the invention includes all such variations and modifications. The invention also includes all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations of any two or more of said steps or features.

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## SEQUENCE LISTING

## (1) GENERAL INFORMATION:

(i) APPLICANT: COMMONWEALTH SCIENTIFIC AND INDUSTRIAL  
RESEARCH ORGANISATION AND THE AUSTRALIAN NATIONAL UNIVERSITY

(ii) TITLE OF INVENTION: PLANT PROMOTER ACTIVATED BY  
FUNGAL INFECTION

(iii) NUMBER OF SEQUENCES: 7

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## (v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk  
(B) COMPUTER: IBM PC compatible  
(C) OPERATING SYSTEM: PC-DOS/MS-DOS  
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

## (vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER: PCT International  
(B) FILING DATE: 03-MAY-1996

## (vii) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER: AU PN 2834/95  
(B) FILING DATE: 05-MAY-1995

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## (2) INFORMATION FOR SEQ ID NO:1:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1853 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: DNA (genomic)

## (ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 54..1706

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

|   |     |
|---|-----|
| ATCAGAGGTC GCCATAGAGA TCATTTCAC TCCAATCAAG CCCTCTGACC ATC ATG   | 56  |
| Met   |     |
| 1   |     |
| TAC AGG CCA CTT GTT GCC AGG TTG CTG CGA GAC AGC GTC GCT ACC CGA | 104 |
| Tyr Arg Pro Leu Val Ala Arg Leu Leu Arg Asp Ser Val Ala Thr Arg |     |
| 5 10 15   |     |
| AAG GGC TCG TCC CAT TTC GCC CGG AGG TTT TCT CAT TCT TTG CCC TTC | 152 |
| Lys Gly Ser Ser His Phe Ala Arg Arg Phe Ser His Ser Leu Pro Phe |     |
| 20 25 30  |     |
| GCG ACC GTA GAT GCG GAG GAG CTA TCT GGT GCT AAA CCA GCT GAA GTG | 200 |
| Ala Thr Val Asp Ala Glu Glu Leu Ser Gly Ala Lys Pro Ala Glu Val |     |
| 35 40 45  |     |
| CTT AAC TTG GTT CAG GGG AAT TGG GGA GGT TCT TCC AGT TGG CAC ACG | 248 |
| Leu Asn Leu Val Gln Gly Asn Trp Gly Gly Ser Ser Ser Trp His Thr |     |
| 50 55 60 65   |     |
| GTG GTT GAT CCT TTA AAC GGA GAA CCG TTT ATC AAA GTT GCT GAA GTA | 296 |
| Val Val Asp Pro Leu Asn Gly Glu Pro Phe Ile Lys Val Ala Glu Val |     |
| 70 75 80  |     |
| GAC GAG ACA GAA ATC AAG CCA TTT GTG GAG AGC TTG TCC AAG TGC CCT | 344 |
| Asp Glu Thr Glu Ile Lys Pro Phe Val Glu Ser Leu Ser Lys Cys Pro |     |
| 85 90 95  |     |
| AAA CAT GGA CTG CAC AAC CCC TTT AAG TCG CCT GAG AGG TAT CTT CTG | 392 |
| Lys His Gly Leu His Asn Pro Phe Lys Ser Pro Glu Arg Tyr Leu Leu |     |
| 100 105 110   |     |
| TAT GGG GAC ATA TCT ACA AAG GCA GGA CAC ATG CTT TCC ATA CCA AAG | 440 |
| Tyr Gly Asp Ile Ser Thr Lys Ala Gly His Met Leu Ser Ile Pro Lys |     |
| 115 120 125   |     |
| GTG TCG GAG TTC TTT GCA AGG CTA ATA CAA AGA GTT GCC CCG AAG AGT | 488 |
| Val Ser Glu Phe Phe Ala Arg Leu Ile Gln Arg Val Ala Pro Lys Ser |     |
| 130 135 140 145   |     |

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|   |      |
|---|------|
| TAC CAC CAG GCT CTT GGT GAA GTT CAA GTC ACC CAG AAG TTT TTT GAG<br>Tyr His Gln Ala Leu Gly Glu Val Gln Val Thr Gln Lys Phe Phe Glu<br>150 155 160     | 536  |
| AAC TTC ACT GGT GAT CAG GTT CGT TTC TTG GCA AGA TCA TTT GGA GTG<br>Asn Phe Thr Gly Asp Gln Val Arg Phe Leu Ala Arg Ser Phe Gly Val<br>165 170 175     | 584  |
| CCG GGA AAC CAT CTT GGT CAG CAA AGT AAT GGC TTC CGA TGG CCT TTT<br>Pro Gly Asn His Leu Gly Gln Gln Ser Asn Gly Phe Arg Trp Pro Phe<br>180 185 190     | 632  |
| GGT CCT GTT GCA ATA ATC ACT CCA TTC AAT TTC CCA CTA GAG ATT CCG<br>Gly Pro Val Ala Ile Ile Thr Pro Phe Asn Phe Pro Leu Glu Ile Pro<br>195 200 205     | 680  |
| GTT CTT CAG TTG ATG GGT GCT TTA TAC ATG GGC AAC AAA CCC CTT CTT<br>Val Leu Gln Leu Met Gly Ala Leu Tyr Met Gly Asn Lys Pro Leu Leu<br>210 215 220 225 | 728  |
| AAA GTT GAT AGC AAG GTG TCC ATT GTT ATG GAA CAA ATG ATG AGA CTA<br>Lys Val Asp Ser Lys Val Ser Ile Val Met Glu Gln Met Met Arg Leu<br>230 235 240     | 776  |
| CTT CAC TAT TGC GGT TTG CCT GTG GGA GAT GCT GAC TTT GTC AAC TCG<br>Leu His Tyr Cys Gly Leu Pro Val Gly Asp Ala Asp Phe Val Asn Ser<br>245 250 255     | 824  |
| GAT GGG AAG GCT ATG AAC AAG ATA CTA CTG GAG GCT AAT CCC CGG ATG<br>Asp Gly Lys Ala Met Asn Lys Ile Leu Leu Glu Ala Asn Pro Arg Met<br>260 265 270     | 872  |
| ACA TTG TTT ACT GGT AGC TCA AGA GTT GCG GAG AAG TTG GCT CTT GAC<br>Thr Leu Phe Thr Gly Ser Ser Arg Val Ala Glu Lys Leu Ala Leu Asp<br>275 280 285     | 920  |
| TTA AAG GGC CGC ATC AAG TTG GAA GAT GCA GGA TTT GAC TGG AAA ATT<br>Leu Lys Gly Arg Ile Lys Leu Glu Asp Ala Gly Phe Asp Trp Lys Ile<br>290 295 300 305 | 968  |
| CTA GGG CCT GAT GTC AAT GAG GCA GAC TAT GTT GCT TGG GTG TGT GAC<br>Leu Gly Pro Asp Val Asn Glu Ala Asp Tyr Val Ala Trp Val Cys Asp<br>310 315 320     | 1016 |
| CAA GAT GCA TAT GCA TGT AGT GGT CAG AAG TGC TCT GCA CAA TCC ATT<br>Gln Asp Ala Tyr Ala Cys Ser Gly Gln Lys Cys Ser Ala Gln Ser Ile<br>325 330 335     | 1064 |
| CTA TTC ATG CAC GAG AAC TGG GCT GCT ACT CCA CTC ATT TCG AGA TTG<br>Leu Phe Met His Glu Asn Trp Ala Ala Thr Pro Leu Ile Ser Arg Leu<br>340 345 350     | 1112 |
| AAG GAG CTT GCA GAG AGG AGA AAG TTG GAA GAT CTA ACT GTT GGC CCT<br>Lys Glu Leu Ala Glu Arg Arg Lys Leu Glu Asp Leu Thr Val Gly Pro<br>355 360 365     | 1160 |
| GTC CTC ACT GTT ACC ACC GAA GCG ATG CTG GAT CAC CTG AAC AAG TTG<br>Val Leu Thr Val Thr Thr Glu Ala Met Leu Asp His Leu Asn Lys Leu<br>370 375 380 385 | 1208 |

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|   |      |
|---|------|
| CTT CAG ATA CCG GGA GCT AAG CTG CTC TTT GGC GGC AAG CCT CTG GAG<br>Leu Gln Ile Pro Gly Ala Lys Leu Leu Phe Gly Gly Lys Pro Leu Glu<br>390 395 400     | 1256 |
| AAT CAT ACC ATT CCA TCC ATA TAT GGT GCC GTG AAA CCA ACA GCC GTG<br>Asn His Thr Ile Pro Ser Ile Tyr Gly Ala Val Lys Pro Thr Ala Val<br>405 410 415     | 1304 |
| TAT GTC CCT CTG GAA GAA ATT CTG AAA GTG AGT AAC TAT GAA CTT GTT<br>Tyr Val Pro Leu Glu Glu Ile Leu Lys Val Ser Asn Tyr Glu Leu Val<br>420 425 430     | 1352 |
| ACA AAG GAA ATC TTC GGA CCA TTC CAG GTT GTA ACG GAG TAC AAG AAC<br>Thr Lys Glu Ile Phe Gly Pro Phe Gln Val Val Thr Glu Tyr Lys Asn<br>435 440 445     | 1400 |
| AGT CAA CTT CCT ATG GTT CTG GAA GCT TTG GAG AGG ATG CAC GCA CAT<br>Ser Gln Leu Pro Met Val Leu Glu Ala Leu Glu Arg Met His Ala His<br>450 455 460 465 | 1448 |
| TTA ACA GCT GCT GTA GTT TCG AAC GAT CAG CTG TTT TTG CAG GAA GTC<br>Leu Thr Ala Ala Val Val Ser Asn Asp Gln Leu Phe Leu Gln Glu Val<br>470 475 480     | 1496 |
| ATC GGG AAC ACT GTG AAT GGC ACA ACT TAT GCC GGG TTG CGA GCA AGA<br>Ile Gly Asn Thr Val Asn Gly Thr Thr Tyr Ala Gly Leu Arg Ala Arg<br>485 490 495     | 1544 |
| ACG ACA GGA GCT CCG CAG AAT CAT TGG TTT GGA CCA GCT GGA GAC CCG<br>Thr Thr Gly Ala Pro Gln Asn His Trp Phe Gly Pro Ala Gly Asp Pro<br>500 505 510     | 1592 |
| AGA GGT GCA GGG ATT GGA ACA CCA GAA GCC ATT AAA CTT GTC TGG TCT<br>Arg Gly Ala Gly Ile Gly Thr Pro Glu Ala Ile Lys Leu Val Trp Ser<br>515 520 525     | 1640 |
| TGC CAC CGA GAG ATC ATT TAC GAT ATC GGC CCT GTA TCA CAC CAT TGG<br>Cys His Arg Glu Ile Ile Tyr Asp Ile Gly Pro Val Ser His His Trp<br>530 535 540 545 | 1688 |
| GAA ATT CCT CCA TCC ACT TAGAGAGAGA GTGAGAGATT TGTAAACTG<br>Glu Ile Pro Pro Ser Thr<br>550   | 1736 |
| TTGAGATGTA GCTGACTGAT CCATGTATCA GAAGTAGGCA TTCATCAGCC CGTGTACCGT   | 1796 |
| TACTTTCTAC GAATAAAAAAT GCCGGGAAGT CTGTAGATCA AAAAAAAAAA AAAAAA  | 1853 |

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## (2) INFORMATION FOR SEQ ID NO:2:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 551 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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Met Tyr Arg Pro Leu Val Ala Arg Leu Leu Arg Asp Ser Val Ala Thr
 1             5             10             15
Arg Lys Gly Ser Ser His Phe Ala Arg Arg Phe Ser His Ser Leu Pro
 20             25             30
Phe Ala Thr Val Asp Ala Glu Glu Leu Ser Gly Ala Lys Pro Ala Glu
 35             40             45
Val Leu Asn Leu Val Gln Gly Asn Trp Gly Gly Ser Ser Ser Trp His
 50             55             60
Thr Val Val Asp Pro Leu Asn Gly Glu Pro Phe Ile Lys Val Ala Glu
 65             70             75             80
Val Asp Glu Thr Glu Ile Lys Pro Phe Val Glu Ser Leu Ser Lys Cys
 85             90             95
Pro Lys His Gly Leu His Asn Pro Phe Lys Ser Pro Glu Arg Tyr Leu
100             105             110
Leu Tyr Gly Asp Ile Ser Thr Lys Ala Gly His Met Leu Ser Ile Pro
115             120             125
Lys Val Ser Glu Phe Phe Ala Arg Leu Ile Gln Arg Val Ala Pro Lys
130             135             140
Ser Tyr His Gln Ala Leu Gly Glu Val Gln Val Thr Gln Lys Phe Phe
145             150             155             160
Glu Asn Phe Thr Gly Asp Gln Val Arg Phe Leu Ala Arg Ser Phe Gly
165             170             175
Val Pro Gly Asn His Leu Gly Gln Gln Ser Asn Gly Phe Arg Trp Pro
180             185             190
Phe Gly Pro Val Ala Ile Ile Thr Pro Phe Asn Phe Pro Leu Glu Ile
195             200             205
Pro Val Leu Gln Leu Met Gly Ala Leu Tyr Met Gly Asn Lys Pro Leu
210             215             220
Leu Lys Val Asp Ser Lys Val Ser Ile Val Met Glu Gln Met Met Arg
225             230             235             240
Leu Leu His Tyr Cys Gly Leu Pro Val Gly Asp Ala Asp Phe Val Asn
245             250             255

```

- 50 -

Ser Asp Gly Lys Ala Met Asn Lys Ile Leu Leu Glu Ala Asn Pro Arg  
 260 265 270  
 Met Thr Leu Phe Thr Gly Ser Ser Arg Val Ala Glu Lys Leu Ala Leu  
 275 280 285  
 Asp Leu Lys Gly Arg Ile Lys Leu Glu Asp Ala Gly Phe Asp Trp Lys  
 290 295 300  
 Ile Leu Gly Pro Asp Val Asn Glu Ala Asp Tyr Val Ala Trp Val Cys  
 305 310 315 320  
 Asp Gln Asp Ala Tyr Ala Cys Ser Gly Gln Lys Cys Ser Ala Gln Ser  
 325 330 335  
 Ile Leu Phe Met His Glu Asn Trp Ala Ala Thr Pro Leu Ile Ser Arg  
 340 345 350  
 Leu Lys Glu Leu Ala Glu Arg Arg Lys Leu Glu Asp Leu Thr Val Gly  
 355 360 365  
 Pro Val Leu Thr Val Thr Thr Glu Ala Met Leu Asp His Leu Asn Lys  
 370 375 380  
 Leu Leu Gln Ile Pro Gly Ala Lys Leu Leu Phe Gly Gly Lys Pro Leu  
 385 390 395 400  
 Glu Asn His Thr Ile Pro Ser Ile Tyr Gly Ala Val Lys Pro Thr Ala  
 405 410 415  
 Val Tyr Val Pro Leu Glu Glu Ile Leu Lys Val Ser Asn Tyr Glu Leu  
 420 425 430  
 Val Thr Lys Glu Ile Phe Gly Pro Phe Gln Val Val Thr Glu Tyr Lys  
 435 440 445  
 Asn Ser Gln Leu Pro Met Val Leu Glu Ala Leu Glu Arg Met His Ala  
 450 455 460  
 His Leu Thr Ala Ala Val Val Ser Asn Asp Gln Leu Phe Leu Gln Glu  
 465 470 475 480  
 Val Ile Gly Asn Thr Val Asn Gly Thr Thr Tyr Ala Gly Leu Arg Ala  
 485 490 495  
 Arg Thr Thr Gly Ala Pro Gln Asn His Trp Phe Gly Pro Ala Gly Asp  
 500 505 510  
 Pro Arg Gly Ala Gly Ile Gly Thr Pro Glu Ala Ile Lys Leu Val Trp  
 515 520 525  
 Ser Cys His Arg Glu Ile Ile Tyr Asp Ile Gly Pro Val Ser His His  
 530 535 540  
 Trp Glu Ile Pro Pro Ser Thr  
 545 550

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## SEQUENCE LISTING

## (2) INFORMATION FOR SEQ ID NO:3:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 347 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: DNA (genomic)

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

|   |     |
|---|-----|
| ATAAACCTCC TATGACTTAC CGAGCAAATG CGGCTGCGGA ACCACTCGAA TCTACCACTC | 60  |
| TTGCATCAAA ACCACCGCCG TAGCTGCCGA CGTTAAAGTA TCCAGAGATA GCAAGTGACG | 120 |
| AGGCAGTGAA GTTAGTAGAG GAAACAGAAA CTGCAGTAAA ATCATTTCAA ATAACAGAGA | 180 |
| GAGAAGAGTT AGCGGCAAAG AGAAGGAGAC TACCATGGAA ACAAAGCTTG AAAACGAAGG | 240 |
| AAAGAGGAAT CGCAAAGAA GAGAGCGATC TTAAAGCTGC ATTTGGCCTC CCTTTCGCGG  | 300 |
| TGTGTTCACT CTCCTCTAT CTCTACTTGC GTCTTGTTT CTCGGCA                 | 347 |

## (2) INFORMATION FOR SEQ ID NO:4:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 161 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: DNA (genomic)

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

ATAAACCTCC ATCACTACCT CTAAAGCTGC ACGGCTGACA CCGCAACACC AAATAACTTG       60  
CCACATTTTC TCTCTATCCA AATCCAAAAT CGACGTCTCT TTCTCCTCCT CATCACTGAG       120  
TTTGTTTCATA CTGCCCCAAC CAAAAGCTTG GTACTTTTAG C                       161

(2) INFORMATION FOR SEQ ID NO:5:

- (i) SEQUENCE CHARACTERISTICS:  
  (A) LENGTH: 21 base pairs  
  (B) TYPE: nucleic acid  
  (C) STRANDEDNESS: single  
  (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA ( degenerate oligonucleotide for which R=purine,  
Y=pyrimidine, I=inosine)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

AARGARAT(Y/A)T TYGGICCITT Y       21

(2) INFORMATION FOR SEQ ID NO:6:

- (i) SEQUENCE CHARACTERISTICS:  
  (A) LENGTH: 21 base pairs  
  (B) TYPE: nucleic acid  
  (C) STRANDEDNESS: single  
  (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA ( degenerate oligonucleotide for which R=purine,  
Y=pyrimidine, I=inosine)

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

AT(Y/A)ACICCIT TYAAYTTYCC I 21

(2) INFORMATION FOR SEQ ID NO:7:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 24 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA ( degenerate oligonucleotide for which R=purine, Y=pyrimidine, I=inosine)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

TGY(A/T) (G/C) IGGIC ARAARTGY(A/T) (G/C) IGGI . 24

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**CLAIMS:**

1. An isolated nucleic acid molecule comprising a sequence of nucleotides which is capable of conferring, activating, enhancing or otherwise increasing the expression of a structural gene in response to a susceptible interaction between a host plant and a fungal pathogen.
2. An isolated nucleic acid molecule according to claim 1, originating from a crop plant selected from the list comprising flax, maize, rice, barley, rye and oats, amongst others.
3. An isolated nucleic acid molecule according to claim 1, wherein said nucleic acid molecule is genetically-linked *in vivo* as hereinbefore defined, to an SR structural gene which is at least 40% identical to the nucleotide sequence set forth in SEQ ID NO: 1 or a complementary strand thereof.
4. An isolated nucleic acid molecule according to claim 1, wherein said nucleic acid molecule is genetically-linked *in vivo* as hereinbefore defined, to an SR structural gene which is at least 60-65% identical to the nucleotide sequence set forth in SEQ ID NO: 1 or a complementary strand thereof.
5. An isolated nucleic acid molecule according to claim 1, wherein said nucleic acid molecule is genetically-linked *in vivo* as hereinbefore defined, to an SR structural gene which is at least 70-75% identical to the nucleotide sequence set forth in SEQ ID NO: 1 or a complementary strand thereof.
6. An isolated nucleic acid molecule according to claim 1, wherein said nucleic acid molecule is genetically-linked *in vivo* as hereinbefore defined, to an SR structural gene which is at least 80-90% identical to the nucleotide sequence set forth in SEQ ID NO: 1 or a complementary strand thereof.

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7. An isolated nucleic acid molecule according to claim 1, wherein said nucleic acid molecule is genetically-linked *in vivo* as hereinbefore defined, to an SR structural gene which hybridises under at least low stringency conditions to the nucleotide sequence set forth in SEQ ID NO: 1 or to a complementary strand thereof.

8. An isolated nucleic acid molecule according to claim 7, wherein said SR structural gene further comprises a sequence of nucleotides which is at least 40% identical to SEQ ID NO: 1 or a complementary strand thereof.

9. An isolated nucleic acid molecule according to claim 7, wherein said SR structural gene further comprises a sequence of nucleotides which is at least 60-65% identical to SEQ ID NO: 1 or a complementary strand thereof.

10. An isolated nucleic acid molecule according to claim 7, wherein said SR structural gene further comprises a sequence of nucleotides which is at least 70-75% identical to SEQ ID NO: 1 or a complementary strand thereof.

11. An isolated nucleic acid molecule according to claim 7, wherein said SR structural gene further comprises a sequence of nucleotides which is at least 80-90% identical to SEQ ID NO: 1 or a complementary strand thereof.

12. An isolated nucleic acid molecule according to claim 1, wherein said nucleic acid molecule comprises a sequence of nucleotides of a least 2 kilobases in length including at the 5' end the sequence of nucleotides set forth in SEQ ID NO: 3 or a homologue, analogue or derivative thereof and at the 3' end the sequence of nucleotides set forth in SEQ ID NO: 4 or a homologue, analogue or derivative thereof.

13. An isolated nucleic acid molecule according to claim 12, further capable of hybridising under at least low stringency conditions to the Flax *FisI* gene promoter contained in the microorganism deposited under AGAL Accession No. N96/027087.

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14. An isolated nucleic acid molecule according to claim 13, wherein said nucleic acid molecule is of plant origin.
15. An isolated nucleic acid molecule according to claim 14, wherein the plant is a crop plant selected from the list comprising flax, maize, barley, rice, rye and oats, amongst others.
16. An isolated nucleic acid molecule which comprises an SR structural gene as hereinbefore defined.
17. An isolated nucleic acid molecule according to claim 16, wherein said nucleic acid molecule originates from a crop plant selected from the list comprising flax, maize, rice, barley, rye and oats, amongst others.
18. An isolated nucleic acid molecule according to claim 16, wherein said SR structural gene is at least 40% identical to the sequence set forth in SEQ ID NO:1 or a homologue, analogue or derivative thereof.
19. An isolated nucleic acid molecule according to claim 16, wherein said SR structural gene further comprises a sequence of nucleotides which is at least 60-65% identical to SEQ ID NO: 1 or a complementary strand thereof.
20. An isolated nucleic acid molecule according to claim 16, wherein said SR structural gene further comprises a sequence of nucleotides which is at least 70-75% identical to SEQ ID NO: 1 or a complementary strand thereof.
21. An isolated nucleic acid molecule according to claim 16, wherein said SR structural gene further comprises a sequence of nucleotides which is at least 80-90% identical to SEQ ID NO: 1 or a complementary strand thereof.
22. An isolated nucleic acid molecule according to claim 16, wherein said nucleic acid

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molecule is capable of hybridising under at least low stringency conditions to the nucleotide sequence set forth in SEQ ID NO: 1 or to a complementary strand thereof.

23. An isolated nucleic acid molecule comprising a sequence of nucleotides which encodes or is complementary to a sequence which encodes an SR gene product as hereinbefore defined.
24. An isolated nucleic acid molecule according to claim 23, wherein said SR gene product comprises an amino acid sequence which is at least 40% identical to the polypeptide set forth in SEQ ID NO: 2.
25. An isolated nucleic acid molecule according to claim 24, originating from a crop plant selected from the list comprising flax, maize, rice, barley, rye and oats, amongst others.
26. An isolated nucleic acid molecule according to claim 25, wherein if said nucleic acid molecule originates from maize it encodes the *MisI* polypeptide.
27. An isolated nucleic acid molecule according to claim 25, wherein if said nucleic acid molecule originates from flax it encodes the *FisI* polypeptide.
28. An isolated nucleic acid molecule capable of hybridising under at least low stringency conditions to the flax *FisI* promoter sequence contained in the microorganism deposited under AGAL Accession No.N96/027087.
29. An isolated nucleic acid primer molecule of at least 10 contiguous nucleotides in length derived from a flax *FisI* nucleotide sequence set forth in any one of SEQ ID NOS: 1, 3, 4, 5, 6 OR 7.
30. A genetic construct which comprises a nucleic acid molecule according to any one of claims 1 to 15.

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31. A genetic construct according to claim 30, wherein said nucleic acid molecule is operably linked to a structural gene.
32. A genetic construct according to claim 31, wherein said structural gene is a reporter gene.
33. A genetic construct according to claim 32, wherein said reporter gene is selected from the list comprising GUS, chloramphenicol acetyltransferase and firefly luciferase genes.
34. A genetic construct deposited under AGAL accession no. N96/027087.
35. A genetic construct according to claim 31 wherein said structural gene comprises a nucleotide sequence which is at least 40% identical to SEQ ID NO: 1 or a homologue, analogue or derivative thereof.
36. A genetic construct according to claim 31, wherein said structural gene is a cytotoxin gene.
37. A genetic construct according to claim 36, wherein said cytotoxin gene is a barnase or other ribonuclease gene.
38. A genetic construct according to claim 30, wherein said nucleic acid molecule is operably linked to a ribozyme, antisense or co-suppression molecule which is capable of inhibiting the expression of a plant gene required for viability of a plant cell.
39. A transgenic plant transformed with a genetic construct according to claim 30.
40. A transgenic plant transformed with a genetic construct according to claim 31.
41. A transgenic plant transformed with a genetic construct according to claim 32.

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42. A transgenic plant transformed with a genetic construct according to claim 33.
43. A transgenic plant transformed with a genetic construct according to claim 34.
44. A transgenic plant transformed with a genetic construct according to claim 35.
45. A transgenic plant transformed with a genetic construct according to claim 36.
46. A transgenic plant transformed with a genetic construct according to claim 37.
47. A transgenic plant transformed with a genetic construct according to claim 38.
48. A genetic construct comprising a nucleic acid molecule according to any one of claims 16 to 27.
49. A recombinant SR gene product comprising a sequence of amino acids which is at least 40% identical to SEQ ID NO: 2.
50. A recombinant SR gene product according to claim 49 further comprising at least one of the amino acid sequence motifs selected from the list comprising GPL, GSG, GQG and EEP as hereinbefore defined in Table 2.
51. An isolated antibody molecule capable of binding to the recombinant SR gene product of claim 49 or 50.
52. A method of producing a plant with improved resistance to the spread of a fungal pathogen in a susceptible interaction, said method comprising the steps of transforming a plant cell with a genetic construct according to any one of claims 36 to 38 and regenerating a whole plant therefrom.

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53. A method according to claim 52, wherein said plant and respective fungal pathogen is selected from the list of pathogen: host plant combinations comprising:

| <b>PATHOGEN</b>                             | <b>HOST PLANT</b>       |
|---|-------------------------|
| <i>Puccinia graminis</i>                    | wheat, barley and rye   |
| <i>Puccinia striiformis</i>                 | wheat and rye           |
| <i>Puccinia recondita</i>                   | rye and wheat           |
| <i>Puccinia hordei</i>                      | barley                  |
| <i>Puccinia coronata</i>                    | oat                     |
| <i>Puccinia sorghi</i>                      | maize                   |
| <i>Puccinia polysora</i>                    | maize                   |
| <i>Puccinia purpurea</i>                    | sorghum                 |
| <i>Puccinia sacchari</i>                    | sugar cane              |
| <i>Puccinia kuehnii</i>                     | sugar cane              |
| <i>Puccinia arachidis</i>                   | peanut                  |
| <i>Puccinia stachmanii</i>                  | cotton                  |
| <i>Uromyces striatus medicaginis</i>        | alfalfa                 |
| <i>Uromyces phaseoli</i>                    | <i>phaseolus</i> beans  |
| <i>Hemileia vastatrix</i>                   | coffee rust             |
| <i>Melampsora lini</i>                      | flax                    |
| <i>Gymnosporangium juniperi-virginianae</i> | cedar and apple         |
| <i>Cronartium ribicola</i>                  | white pine              |
| <i>Cronartium fusiforme</i>                 | loblolly and slash pine |

54. A method according to claim 53, wherein said plant is selected from the list comprising flax, maize, wheat, barley, oats, rye and rice.

55. A method of detecting infection of a plant by a fungal pathogen which is capable of forming a susceptible interaction therewith, said method comprising the step of detecting

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increased expression of an SR structural gene in said plant.

56. A method according to claim 55, wherein said step of detecting increased expression is performed by contacting a nucleic acid molecule derived from said plant with a second nucleic acid molecule comprising the nucleotide sequence set forth in SEQ ID NO:1 or a fragment of at least 10 contiguous nucleotides in length derived therefrom for a time and under conditions sufficient to allow a double-stranded nucleic acid molecule to form.

57. A method according to claim 56, wherein said second nucleic acid molecule is labelled with a reporter molecule.

58. A method according to claim 57, further comprising the step of detecting the amount of bound reporter molecule.

59. A method according to claim 55 when used for the detection of an infection in a plant by a fungal pathogen, wherein said plant and respective fungal pathogen is selected from the list of pathogen: host plant combinations comprising:

**PATHOGEN**

*Puccinia graminis*  
*Puccinia striiformis*  
*Puccinia recondita*  
*Puccinia hordei*  
*Puccinia coronata*  
*Puccinia sorghi*  
*Puccinia polysora*  
*Puccinia purpurea*  
*Puccinia sacchari*  
*Puccinia kuehnii*  
*Puccinia arachidis*

**HOST PLANT**

wheat, barley and rye  
wheat and rye  
rye and wheat  
barley  
oat  
maize  
maize  
sorghum  
sugar cane  
sugar cane  
peanut

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|   |                         |
|---|-------------------------|
| <i>Puccinia stachmanii</i>                  | cotton                  |
| <i>Uromyces striatus medicaginis</i>        | alfalfa                 |
| <i>Uromyces phaseoli</i>                    | <i>phaseolus</i> beans  |
| <i>Hemileia vastatrix</i>                   | coffee rust             |
| <i>Melampsora lini</i>                      | flax                    |
| <i>Gymnosporangium juniperi-virginianae</i> | cedar and apple         |
| <i>Cronartium ribicola</i>                  | white pine              |
| <i>Cronartium fusiforme</i>                 | loblolly and slash pine |

60. A method according to claim 59, wherein said plant is selected from the list comprising flax, maize, wheat, barley, oats, rye and rice.

61. A progeny plant derived from the transgenic plant according to any one of claims 27 to 33.

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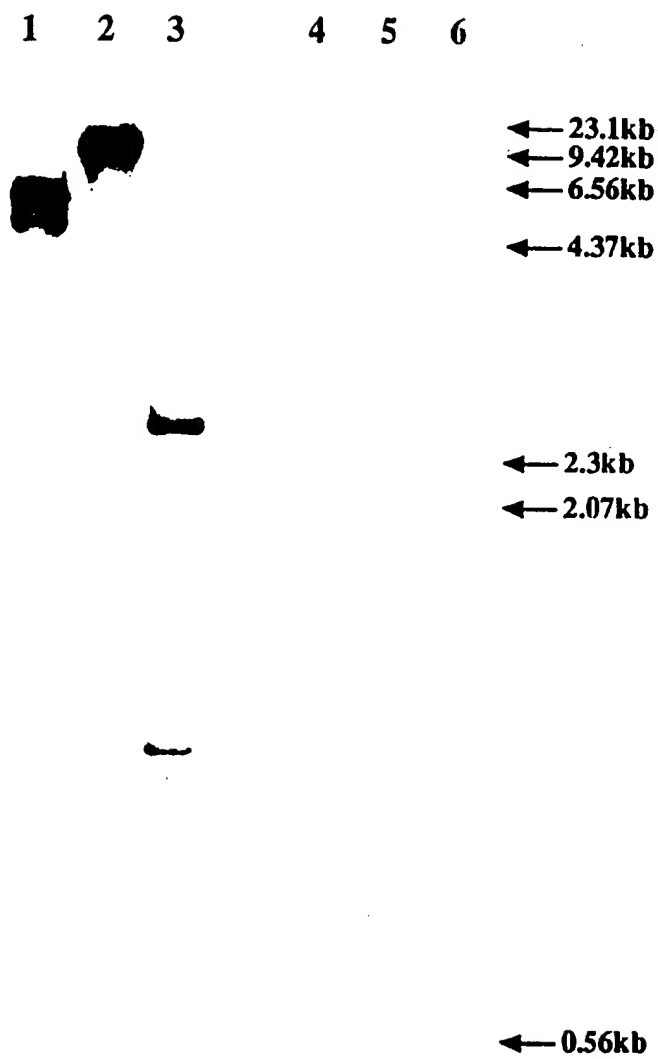


FIGURE 1

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# FIS promoter into BI 101 for transformation of Flax

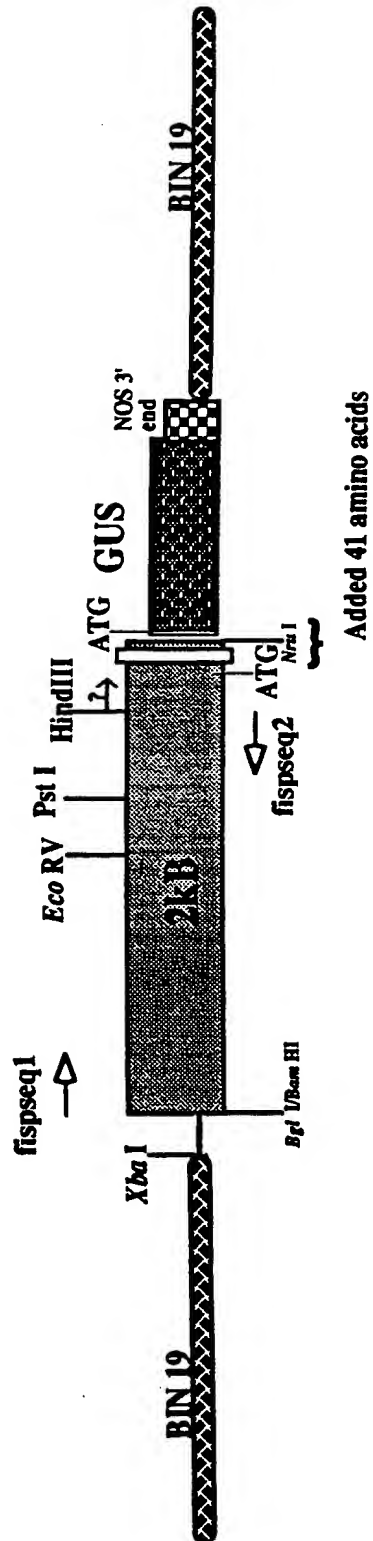


FIGURE 2a

△ areas where the promoter has been sequenced

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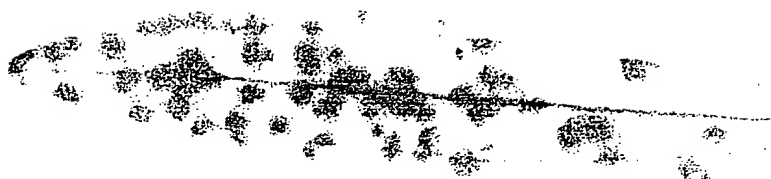


FIGURE 2b

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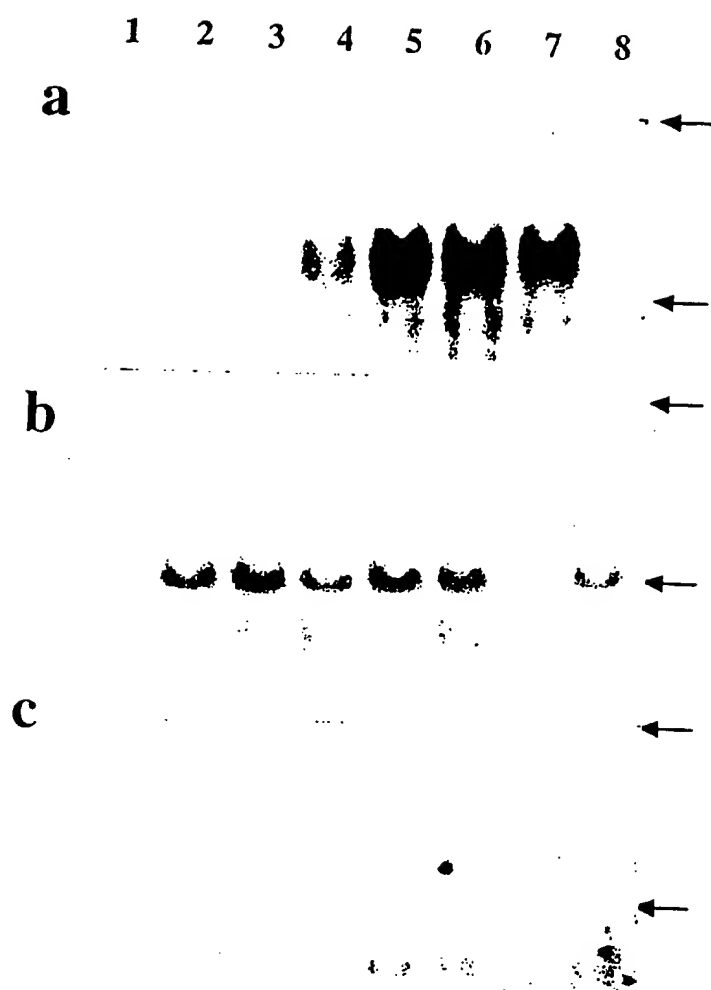


FIGURE 3

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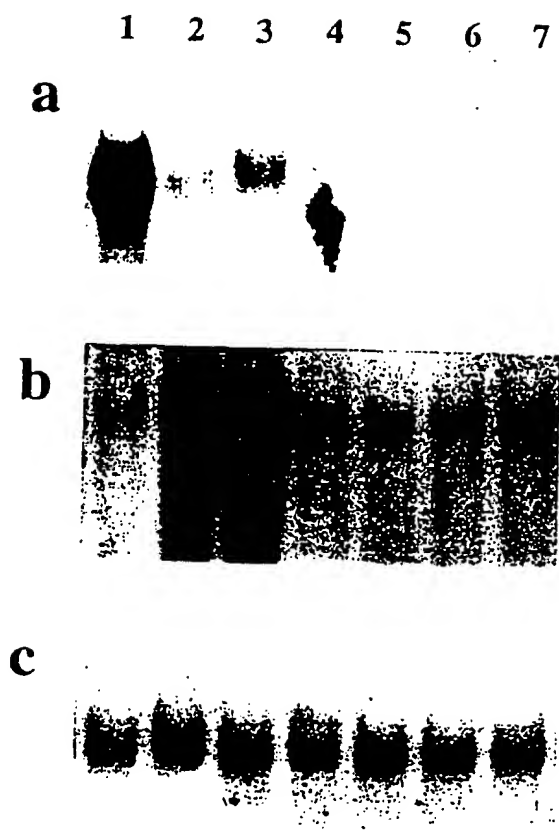


FIGURE 4

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**GPL motif**  
**mis:** NEPLEIPLLQLMGALYMGNKPVLKVD SKVSIVMEQMI RLLHDCGLPAEDMDFINSDGAVM  
 NFPLEIP+LQLMGALYMGNKP+LKVD SKVSIVMEQMI RLLH CGLP D DF+NSDG M  
**fis:** NEPLEIPVLQLMGALYMGNKPLLKVD SKVSIVMEQMI RLLHYCGLPVGDA DFVNSDGKAM  
 203 262

**GSG motif**  
**mis:** NKLLLEANPKMTLETGSSRVAEKL AADLKGRVKLEDA GFDWKILGPDV  
 NK+LLEANP+MTLFTGSSRVAEKL A DLKGR+KLEDA GFDWKILGPDV  
**fis:** NKLLLEANPKMTLFTGSSRVAEKL ALDLKGRIKLEDA GFDWKILGPDV  
 263 310

**active site**  
**mis:** DYVAWVCDQDAYACSGQKCSAQSVLFMHK  
 DYVAWVCDQDAYACSGQKCSAQSVLFMH+  
**fis:** DYVAWVCDQDAYACSGQKCSAQSVLFMH  
 314 342

**EEP motif**  
**mis:** LTVTTEAMLEHMNNLLKIRGSKVLFGEPLANHSIPKIYGAMKPTAVFVPLEEILKSGNFELVTKEIFGP  
 LTVTTEAML+H+N LL+I G+K+LFGG+PL NH+IP IYGA+KPTAV+VPLEEILK N+ELVTKEIFGP  
**fis:** LTVTTEAMLDHLNKL LQIPGAKLLFGGKPLENHTIPSIYGAVKPTAVVVPLEEILKVSNYELVTKEIFGP  
 371 440

FIGURE 5

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*Mis 1* INDUCTION  
(Days after rust inoculation)

|   |   |   |   |   |   |   |
|---|---|---|---|---|---|---|
| 0 | 6 | 5 | 4 | 3 | 2 | 0 |
|---|---|---|---|---|---|---|

23s ↑

18s ↑

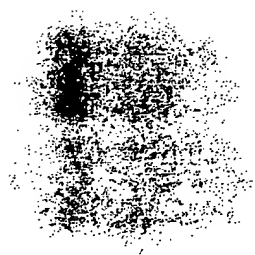
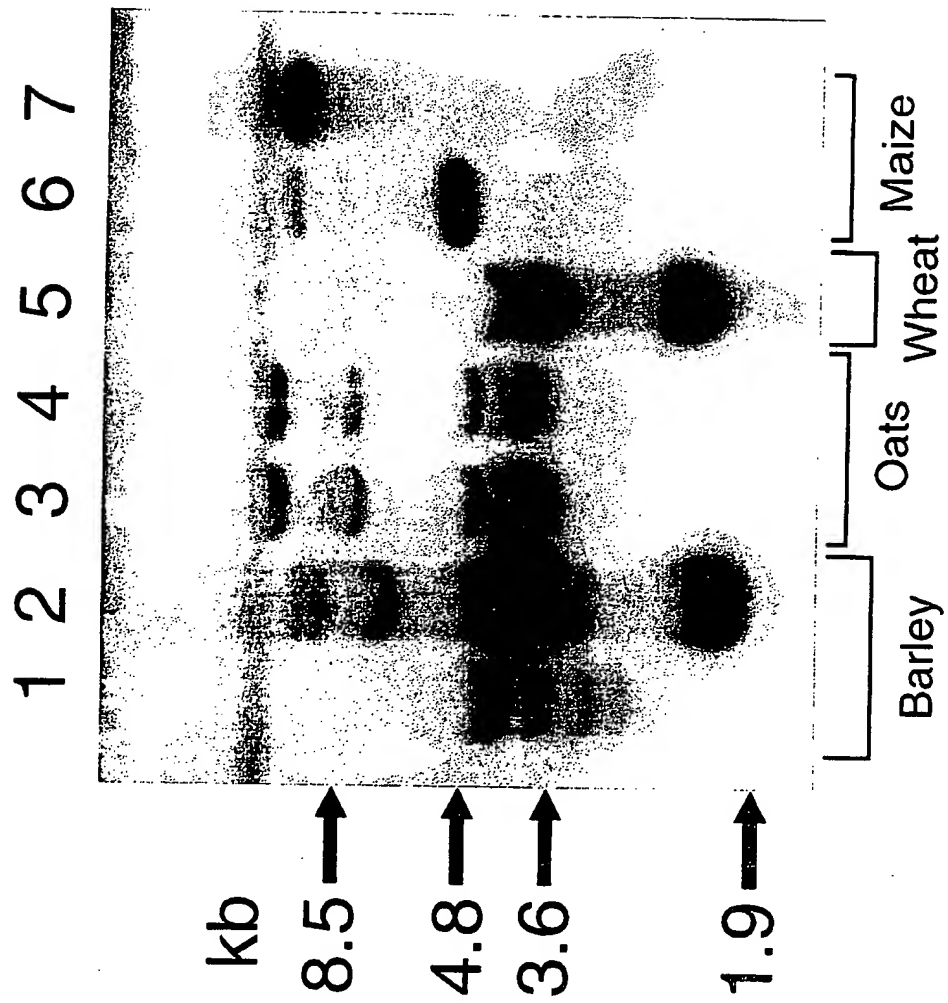


FIGURE 6



**FIGURE 7**

# INTERNATIONAL SEARCH REPORT

International Application No.  
PCT/AU 96/00264

| <b>A. CLASSIFICATION OF SUBJECT MATTER</b>  |  |  |  |   |  |  |   |  |  |   |  |  |
|---|--|--|--|---|--|--|---|--|--|---|--|--|
| Int Cl <sup>6</sup> : C12N 15/11, 15/29 A01H 3/00, 5/00   |  |  |  |   |  |  |   |  |  |   |  |  |
| According to International Patent Classification (IPC) or to both national classification and IPC   |  |  |  |   |  |  |   |  |  |   |  |  |
| <b>B. FIELDS SEARCHED</b>   |  |  |  |   |  |  |   |  |  |   |  |  |
| Minimum documentation searched (classification system followed by classification symbols)<br>WPAT and CHEM ABS<br>See details in electronic database box below.   |  |  |  |   |  |  |   |  |  |   |  |  |
| Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched   |  |  |  |   |  |  |   |  |  |   |  |  |
| Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)<br>DERWENT WPAT, CHEM ABS DATA BASES; KEYWORDS; PUCCINIA, UROMYCES, HEMILEIA, MELAMPSORA, GYMNOSPORANGIUM, CRONARTIUM, FIS (W), 1, MIS (W) REGULAT., CONTROL, INDUC #, SUSCEPIBLE (2N) REACTION #, PLANT #, PROMOTER #, PATHOGEN #, FUNG., HOST (2N) DEFENC.   |  |  |  |   |  |  |   |  |  |   |  |  |
| <b>C. DOCUMENTS CONSIDERED TO BE RELEVANT</b>   |  |  |  |   |  |  |   |  |  |   |  |  |
| Category*   | Citation of document, with indication, where appropriate, of the relevant passages   | Relevant to claim No.  |  |   |  |  |   |  |  |   |  |  |
| X   | AU 75189/94 (VIRGINIA TECH INTELLECTUAL PROPERTIES INC)<br>published 9 February 1995   | 1, 16, 23, 55  |  |   |  |  |   |  |  |   |  |  |
| P,X   | AU 24178/95 (CIBA-GEIGY AG), published 14 December 1995<br>see pages 20-23   | 1  |  |   |  |  |   |  |  |   |  |  |
| X   | The Plant Cell (1989), vol 1, pg 151-158, (Logemann J et al) "5'<br>UPSTREAM SEQUENCES FROM THE WUN 1 GENE ARE RESPONSIBLE<br>FOR GENE ACTIVATION BY WOUNDING IN TRANSGENIC PLANTS."<br>See pages 151, 154 and 155                               | 1, 16, 23, 55  |  |   |  |  |   |  |  |   |  |  |
| <input type="checkbox"/> Further documents are listed in the continuation of Box C <input type="checkbox"/> See patent family annex   |  |  |  |   |  |  |   |  |  |   |  |  |
| <p>* Special categories of cited documents:</p> <table border="0"> <tr> <td>"A" document defining the general state of the art which is not considered to be of particular relevance</td> <td>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</td> </tr> <tr> <td>"E" earlier document but published on or after the international filing date</td> <td>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone</td> </tr> <tr> <td>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</td> <td>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art</td> </tr> <tr> <td>"O" document referring to an oral disclosure, use, exhibition or other means</td> <td>"&amp;" document member of the same patent family</td> </tr> <tr> <td>"P" document published prior to the international filing date but later than the priority date claimed</td> <td></td> </tr> </table> |  |  | "A" document defining the general state of the art which is not considered to be of particular relevance | "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention | "E" earlier document but published on or after the international filing date | "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone | "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) | "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art | "O" document referring to an oral disclosure, use, exhibition or other means | "&" document member of the same patent family | "P" document published prior to the international filing date but later than the priority date claimed |  |
| "A" document defining the general state of the art which is not considered to be of particular relevance  | "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention  |  |  |   |  |  |   |  |  |   |  |  |
| "E" earlier document but published on or after the international filing date  | "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone   |  |  |   |  |  |   |  |  |   |  |  |
| "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)   | "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art |  |  |   |  |  |   |  |  |   |  |  |
| "O" document referring to an oral disclosure, use, exhibition or other means  | "&" document member of the same patent family  |  |  |   |  |  |   |  |  |   |  |  |
| "P" document published prior to the international filing date but later than the priority date claimed  |  |  |  |   |  |  |   |  |  |   |  |  |
| Date of the actual completion of the international search   |  | Date of mailing of the international search report<br><b>21 AUG 1996</b>       |  |   |  |  |   |  |  |   |  |  |
| Name and mailing address of the ISA/AU<br>AUSTRALIAN INDUSTRIAL PROPERTY ORGANISATION<br>PO BOX 200<br>WODEN ACT 2606<br>AUSTRALIA Facsimile No.: (06) 285 3929   |  | Authorized officer<br><br><b>MARK DONAGHEY</b><br>Telephone No.: (06) 283 2156 |  |   |  |  |   |  |  |   |  |  |

| C (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT |  |                       |
|--|--|-----------------------|
| Category*  | Citation of document, with indication, where appropriate, of the relevant passages   | Relevant to claim No. |
| X  | Plant Molecular Biology (1993), vol 22, pg 475-490 (Mohan R et al), DEVELOPMENTAL AND TISSUE-SPECIFIC EXPRESSION OF A TOMATO ANIONIC PEROXIDASE (tap 1) GENE BY A MINIMAL PROMOTER, WITH WOUND AND PATHOGEN INDUCTION BY AN ADDITIONAL 5' -FLANKING REGION".<br>see abstract | 1                     |
| P,X  | Biotechnology (1995), vol 13, pg 1085-1089. (Strittmatter G et al), "INHIBITION OF FUNGAL DISEASE DEVELOPMENT IN PLANTS BY ENGINEERING CONTROLLED CELL DEATH"  | 1, 16, 23, 55         |
| A  | AU 76845/91 (RIJKS LANDBOUW UNIVERSITEIT WAGENINGEN), published 17 October 1991,   |                       |

**INTERNATIONAL SEARCH REPORT**

International Application No.

**PCT/AU 96/00264****Box I**      **Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)**

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
  
2. ☒ Claims Nos.: 3, 4, 5, 6, 8, 9, 10, 11, 18, 19, 20, 21, 24, 35, and 49  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:  
The exact sequence has been searched but not any of the honologies.
  
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a)

**Box II**      **Observations where unity of invention is lacking (Continuation of item 2 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
  
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

**Remark on Protest**

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

Box

Continuation of Box 49

CHEM ABS MYRPLVARLL/YGDESTKAGH/SOSP.

# INTERNATIONAL SEARCH REPORT

## Information on patent family members

International Application No.

PCT/AU 96/00264

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

| Patent Document Cited in Search Report |          |    |         | Patent Family Member |         |    |       |
|--|----------|----|---------|----------------------|---------|----|-------|
| AU                                     | 75189/94 | WO | 9503690 | EP                   | 712273  |    |       |
| AU                                     | 24178/95 | IL | 114018  | WO                   | 9533818 |    |       |
| AU                                     | 76845/91 | EP | 474857  | WO                   | 9115585 | IL | 97736 |
|  |          | JP | 5505110 |                      |         |    |       |
| END OF ANNEX                           |          |    |         |                      |         |    |       |